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Abstracts

ORAL PRESENTATIONS

O.01

Platinum chemotherapy: an oncologic success story

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Cisdiamminedichloroplatinum (cisplatin) first entered clinical trials in 1973. Early results were discouraging with only modest activity in refractory tumors and severe toxicity. Testicular cancer perhaps saved this drug from extinction and cisplatin combination chemotherapy has revolutionized the cure rate of metastatic testis cancer from 5% B.C. (before cisplatin) to the current 80%. A third of a century later, platinum compounds are the cornerstone for chemotherapy regimens in small cell and non-small cell lung cancer, head and neck cancer, esophageal cancer, bladder cancer, cervical cancer, ovarian cancer, thymoma, and osteogenic sarcoma. However, the dramatic success seen in germ cell tumors has never been replicated by platinum compounds or any other cytolytic agent in any other solid tumor. In addition to a very wide spectrum of activity, platinum is also unique in its ability to exhibit synergy with numerous other chemotherapy agents, most notably etoposide and fluorouracil. It also combines well with radiotherapy to improve survival in locally advanced cancers such as cervical cancer, cancer of esophagus, head and neck cancer, and small cell and non-small cell lung cancer. Recent research with platinum compounds have focused on new platinum agents with novel efficacy, such as oxaliplatin in colorectal cancer and satraplatin in prostate cancer, as well as understanding and overcoming mechanisms of platinum resistance.

O.02

Platinum compounds in clinical development
Calvert H
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Abstract not received

O.03

Satraplatin: overview
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Abstract not received

O.04

Satraplatin in prostate cancer
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Abstract not received

O.05

Novel drug delivery systems: ProLindac™ (AP5346)

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ProLindac™ (AP5346) is a macromolecular carrier linked to a bioactive DACH-platinum complex[1]. The macromolecular carrier is a water soluble, biocompatible, 90:10 random copolymer of a N-(2-hydroxypropyl)methacrylamide (HPMA) monomer and a methacrylamide monomer substituted with a triglycyl-amidomalonate group, the linker to the platinum complex. ProLindac™ was designed to act as a prodrug in which the DACH platinum remains inert until preferentially released in the tumor environment. The polymer delivery system takes advantage of the enhanced permeability and retention effect in which tumor neovasculature shows greater permeability to macromolecules than normal tissue, often associated with limited lymphatic and/or capillary drainage. *In vitro* studies demonstrate that the release of DACH-platinum is accelerated in low pH environments[2], such as the extracellular space of hypoxic tumors and the intracellular lysosomal compartment. ProLindac™ produced greater tumor growth inhibition and prolonged growth delay compared to equitoxic doses of oxaliplatin in the B16 melanoma and 2008 ovarian tumor models[3]. ProLindac™ also out-performed oxaliplatin in the HT-29 colorectal, Lewis lung and M5076 cisplatin-resistant tumor models. The first phase I dose escalation clinical trial of ProLindac™ was conducted in solid tumor patients. ProLindac™ was administered in a 1-h IV infusion once per week for 3 of each of the 4 weeks in a cycle[4]. The dose was escalated from 40 to 1,280 mg Pt/m^2. The recommended phase 2 dose using this schedule was 640 mg Pt/m^2. The toxicity profile was characterized by frequent grade 1-2 nausea, vomiting and creatinine elevations, with an absence of grade 3-4 hematotoxicity below 1,280 mg Pt/m^2. There was no evidence of acute neurotoxicity; the total number of cycles given was too few to assess chronic neurotoxicity. Evidence of anti-tumor activity was observed at 640 mg Pt/m^2 and above, with partial responses achieved in patients with melanoma and ovarian cancer. Pharmacokinetic studies were carried out in 26 patients. C_{max} and $AUC_{0\text{-}1wk}$ for total plasma platinum increased linearly with dose. Only a very small fraction of the total platinum was free in the plasma, with the ultrafiltrate Pt $AUC_{0\text{-}1wk}$ representing, on average, only 0.6% of the total Pt AUC. Mean terminal half-life was 72.3 $\pm$ 16.9 h for total Pt and 56.7 $\pm$ 14.7 h for ultrafiltrate Pt at doses $\geq$ 320 mg/m^2. Phase II assessment of ProLindac™ is in progress in patients with recurrent platinum-sensitive advanced ovarian cancer.

References

1. J. Rice, S Howell, Drugs of the Future 2004, 29:561-565.
2. P. Sood, K Thurmond, J. Jacob *et al.*, Bioconj. Chem. 2006, 17:1270 - 1279.
3. J. Rice, J. Gerberich, D. Nowotnik, S. Howell, Clin Cancer Res. 2006, 12:2248-2254.
4. Campone, M. et al. Cancer Chemother Pharmacol, 60:523-533, 2007.

O.06

Physicochemical characteristics and preclinical studies of novel anticancer drug Dicycloplatin

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OBJECTIVE: To investigate the physiochemical characteristics, preclinical in vitro and in vivo antitumor activities as well as acute toxicities of the novel anticancer drug dicycloplatin (DCP).
METHODS: Dicycloplatin was synthesized from platinum (Pt) powder through 10 steps. The crystal structure, physicochemical characteristics, and its water solution stability of DCP were studied. The in vitro cytotoxicity of DCP was tested against the 7 human cancer cell lines including gastric carcinoma BCG-823, non-small cell lung cancer A549, colon cancer HT-29, breast cancer 475A, bladder carcinoma E.J, hepatocellular cancer Bel-7402, and oral carcinoma K.B by MTT method. The in vivo antitumor activity was tested against three mouse tumor models including Lewis lung carcinoma (LLC), liver cancer H22, and sarcoma S180. The acute toxicities of the drug were investigated by single intravenous and intraperitoneal administration of different doses of DCP.
RESULTS: Dicycloplatin is a white color, needle-like crystal. Its chemical structure maintained stable for 6 years in room temperature. The water solubility of dicycloplatin is much better than cisplatin and carboplatin. No dissociation and damages were observed for the drug solution after 3 years at room temperature. DCP demonstrated in vitro cytotoxicity to all 7 human cancer cell lines, especially to gastric, liver, and bladder cancer cell lines, which were more sensitive to the drug with IC_{50} 25-30 μmol.L-^{1}. DCP also showed a significant in vivo antitumor activity against the three mouse tumor models with tumor growth inhibition (TGI) of 67-90% compared to 51-63% of TGI from the positive control drug carboplatin. The acute toxicity profile of DCP (LD_{50} = 210 mg . kg-^{1}) was superior to carboplatin and cisplatin, which were 164 mg.kg-^{1} and 14.27 mg.kg-^{1}, respectively.
CONCLUSION: DCP has a stable chemical structure, good water solubility, and a significant antitumor activity in vitro and in vivo. The studies demonstrated that the antitumor activity of DCP was greater than carboplatin, and the safety profile was much better than carboplation and cisplatin. A phase I clinical trial for dicycloplatin has been successfully completed recently, which showed a good safety in human with lower incidence of gastrointestinal side-effects and hematological toxicities than cisplatin and carboplatin. These results indicate that dicycloplatin represents a new generation of platinum anticancer drug for cancer treatment. A multiple center and randomized phase II clinical trial is underway currently.

O.07

Phase I and phase II clinical trial of novel anticancer drug dicycloplatin in patients with advanced solid malignancies

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OBJECTIVE: The objectives of the open-label phase I and PK study were to characterize the principal toxicities and determine the MTD of dicycloplatin (DCP). The study also sought to determine the recommended dose for phase II trials and detect preliminary evidence of antitumor activity. A multicenter randomized phase II study is ongoing to determine the efficacy and safety of the combination of DCP with paclitaxel in advanced non-small cell lung cancer (NSCLC).
METHODS: In the phase I study, eligible patients had histologic and/or cytologic diagnosis of nonhematologic cancer failed to conventional treatment; life expectancy of $\geq$ 3 months; normal renal, hepatic, and bone marrow function; and an ECOG PS < 2. DCP was administered as a single dose iv infusion over 1 h to these patients. In phase II study, patients with grade IIIB-IV NSCLC were randomized to receive DCP 450 mg/m^2 and paclitaxel 175 mg/m^2 compared to the combination of carboplatin AUC 6 plus paclitaxel every 21 days for 4-6 cycles.
RESULTS: For phase I study, 29 patients (mean age, 50 years; male/female 23:6) enrolled in 8 dose levels at 50, 100, 175, 250, 350, 450, 550, 650/m^2, respectively. The MTD level of DCP was 650 mg/m^2; further dose escalation was precluded by G3/4 toxicities in bone marrow and digestive tract. A total of 10 grade III/IV toxicities were observed (nausea and vomiting, 6; anemia, 2; thrombocytopenia, 2), which occurred in 650 mg/m^2 dose group. The others were grade I/II toxicities. Two heavily pretreated patients with non-small cell lung cancer experienced partial response, and 13 patients had stable disease. The drug exhibited linear pharmacokinetics characterized by distribution half-life of 1.49 ± 0.39 h and a low steady-state apparent volume of distribution (177.88 ± 44.92 L/m^2), which fits 2-compartment pattern. In phase II trial, so far 138 patients were enrolled with 64 (DCP 30 and carboplation 34 cases) evaluable for response. After 2 treatment cycles. DCP plus paclitaxel arm had response rates of PR 30%, SD 67%, PD 3%. Compared to carboplatin plus paclitaxel arm with PR 18%, SD 70%, and PD 12%. Up to date 26 cases (13 each arm) had received 4 cycles of treatment, DCP/paclitaxel arm PR 54%, SD 46% vs carbo/paclitaxel arm PR 38.4%, SD 38.4% and PD 23%. The median duration of disease control and the time to progression have not achived . DCP/paclitaxel combination was safe and generally well tolerated. The incidence of AEs occurred more frequetly in the carboplatin plus paclitaxel group (mainly neutropenia and hypersensitivity).
CONCLUSION: DCP was well tolerated in patients with advanced solid malignancies. The recommended dose of DCP as a single agent is 550 mg/m^2 every 3 weeks. The preliminary data indicated that the efficacy and safety of DCP/paclitaxel are superior to carboplatin/paclitaxel in the 1st line treatment for advanced NSCLC. The multicenter randomized phase II clinical trial is still in progressing.

O.08

Carboplatin nanocapsules: a highly cytotoxic, phospholipid-based formulation of carboplatin

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Platinum-based drugs are widely used in cancer chemotherapy. However, their clinical use is limited by systemic toxicity, rapid blood clearance, and the occurrence of resistance. Our research is aimed at increasing the therapeutic index of these drugs by encapsulation in a lipid formulation. Previously, we developed a method for efficient encapsulation of cisplatin, yielding cisplatin nanocapsules [1, 2]. Cisplatin nanocapsules consist of bean-shaped essentially water-free cisplatin nanoprecipitates surrounded by a single phospholipid bilayer [3], and are very cytotoxic towards a panel of human carcinoma cells.
Carboplatin, a cisplatin-derived anticancer drug with different chemical properties, can be efficiently encapsulated by a similar method [4]. Surprisingly, the carboplatin nanocapsules are structurally different from the cisplatin nanocapsules: carboplatin nanocapsules are small (diameter ≤300 nm) multilamellar spheres consisting of very tightly wrapped bilayers enriched in carboplatin. The carboplatin nanocapsules exhibit a very high cytotoxicity *in vitro*: the IC50 value of carboplatin nanocapsules is up to a 1000-fold lower than that of free carboplatin when tested on a panel of carcinoma cell lines. Cellular platinum-content analysis and confocal fluorescent imaging of the interaction of the carboplatin nanocapsules with IGROV1 cells indicate that the improved cytotoxicity is due to increased platinum accumulation in the cells, resulting from uptake by endocytosis.

References

1. Burger, K. N., Staffhorst, R. W., de Vijlder, H. C., et al. Nanocapsules: lipid-coated aggregates of cisplatin with high cytotoxicity. *Nat Med* 2002; 8: 81-84.
2. de Kroon, A. I., Staffhorst, R. W., Kruijff, B., and Burger, K. N. Cisplatin nanocapsules. *Methods Enzymol* 2005; 391: 118-125.
3. Chupin, V., de Kroon, A. I., and de Kruijff, B. Molecular architecture of nanocapsules, bilayer-enclosed solid particles of Cisplatin. *J Am Chem Soc* 2004; 126: 13816-13821.
4. Hamelers, I. H., van Loenen, E., Staffhorst, R. W., de Kruijff, B., and de Kroon, A. I. Carboplatin nanocapsules: a highly cytotoxic, phospholipid-based formulation of carboplatin. *Mol Cancer Ther* 2006; 5: 2007-2012.

O.09

Assessment of the in vivo antiproliferative activity of a novel platinum nanoparticulate pharmacophore

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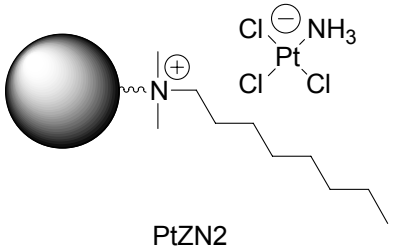
Cisplatin is one of the most effective anticancer agents, but its clinical utility is limited by a number of drawbacks, such as severe toxic side effects and by intrinsic and acquired drug resistance. The "drug targeting and delivery" approach, based on conjugation of cytotoxic agents to molecules having selective access to tumors, has been proposed as an interesting strategy to improve the selectivity and the therapeutic index of platinum derivatives. Attachment to polymeric carriers can significantly alter cellular drug uptake with respect to the free drug, prolong its plasma half-life and, most notably, take advantage of the *Enhanced Permeability and Retention* (EPR) effect that characterizes tumour blood and lymphatic vessels. In the present study, a water-soluble, biocompatible polymer, suitably modified for Pt coordination, was used as selective carrier for the anionic Pt-complex $K[PtCl_3NH_3]$ (PtA), previously selected from a series of charged Pt-derivatives, showing lower cytotoxicity than cisplatin. A nanosphere (ZN2) core-shell-like bioconjugate was obtained from a poly-metacrylate (PMMA) with protruding quaternary ammonium groups, which form ionic couples (PtZN2) with the negatively charged PtA. *In vitro* cytotoxicity studies on B16 murine melanoma cell line, performed by the MTT assay, showed an IC_{50} = 10.47 ± 1.31 ¤M for PtA and an IC_{50} = 0.41 ± 0.14 ¤M for cisplatin (mean ± s.e.m). *In vivo* studies showed that PtA-ZN2, at the estimated maximum tolerated dose (MTD) of 25 mg Pt/Kg/die for 5 days, is significantly more effective than cisplatin, also at its MTD of 5 mg Pt/Kg/die for 5 days, in inhibiting the growth of B16 melanoma in mice. In contrast, treatment with PtA does not affect tumor growth as compared to control treatment. *In vivo* efficacy was found to correlate with Pt intratumor accumulation, as evaluated by ICP-MS following tumor tissue mineralization. PtZN2-treated mice did not show any signs of nephrotoxicity, as evaluated by the measure of creatinine plasma levels. Overall, our results indicate that, in spite of the limited cytotoxicity of PtA, the PtZN2 conjugate has a good therapeutic index and, thus, could be the starting point for the development of novel platinum bioconjugates.



PtZN2

O.10

Studies on new platinum compounds and drug combination

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Widespread use in the clinic and increasing volume of sale of platinum drugs indicate that even in post-genomic age there is a great demand for shot-gun chemotherapy to kill cancerous cells and considerable research effort has been directed in designing novel platinum and other metal compounds with the aim of widening spectrum of activity and reducing side effects. A number of mononuclear *trans*-planaramine platinum(II) complexes of the forms: *trans*-[$Pt(NH_3)LCl_2$] and *trans*-[PtL_2Cl_2 where L stands for a planaramine ligand and multicentred platinum compounds have been synthesized in our laboratory as well. One complex of the form: *trans*-[$Pt(NH_3)LCl_2$] where L is (1,2-α)pyridine (code named YH12) is found to be significantly more active than cisplatin against ovarian $A2780^{cisR}$ cancer cell line. It has a resistance factor of 0.53. A number of tri-nuclear complexes in which the central metal ion is bonded to one or two planaramine ligands have also been synthesized. Five of them code named DH6Cl, DH7Cl, CH9, CH25 and TH1 have been found to be significantly more active than cisplatin in a number of ovarian cancer cell lines. Recently we have carried out studies on synergism between platinum drugs and that between platinum drugs and selected plant-based substances. Results show that YH12, DH6Cl and TH1 show sequence- and dose-dependent synergism in activity with cisplatin in ovarian cancer cell lines. This presentation will provide a brief overview of the work done in our laboratory during the last ten years with special emphasis to rule-breaker platinum compounds and synergism in activity from drug combination including both platinum and non-platinum drugs.

O.11

Synthesis of cisplatin analogues: cytoxic and antitumor activity of bis-amidine and bis-iminoether Pt(II) complexes

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Many analogues of cis-diamminodichloroplatinum(II) (*cisplatin*) have been synthesized with the attempt to overcome either the toxic side effects or to broaden its clinical spectrum of activity [1]. The *cis*- configuration was initially identified as the only active one. However, several *trans*-platinum complexes have been subsequently found to exhibit antitumor-activity. In transplatin, replacement of amine groups by ligands such as sterically hindered planar amines, branched asymmetric aliphatic amines and iminoethers results in an enhancement of its cytotoxicity and significant antitumor activity. New classes of antitumor trasplatinum drugs have been recently reported bearing iminoether non-leaving ligands [2].
Recently, new benzyliminoether derivatives $[PtCl_2\{N(H)=C(OMe)CH_2Ph\}_2]$ of *cis* and *trans* geometry were prepared and characterized by means of elemental analysis, multinuclear NMR, FT-IR techniques and X-ray crystallography. The cytotoxic properties of these new platinum(II) complexes were evaluated in terms of cell growth inhibition against a panel of different types of human cancer cell lines. *Cis*-$[PtCl_2\{E\text{-}N(H)=C(OMe)CH_2Ph\}_2]$ (**1a***EE*) was significantly more potent than cisplatin against all tumor cell lines tested, showing IC_{50} values from about 2- to 17-fold lower than the reference compound. Chemosensitivity tests performed on some human cancer cell line pairs selected for their resistance to cisplatin demonstrated that complex **1a***EE* is able to overcome cisplatin-resistance. Studying the mechanism by which complex **1a***EE* led to cell death, we have found that it induced apoptosis in a dose-dependent manner accompanied by the activation of caspase-3. The antitumor activity of derivative **1a***EE* was also evaluated *in vivo*, against two transplantable tumor models: L1210 leukemia and Lewis lung carcinoma. Chemotreatment with complex **1a***EE* resulted in a remarkable antitumor activity in both tumor models, as measured by prolonged survival and reduced tumor mass compared to control groups. In addition, a cisplatin resistant subline of L1210 (L1210/R), showed a high *in vivo* sensitivity to complex **1a***EE* in terms of treated survivors relative to controls, whereas cisplatin was almost ineffective. *In vivo* studies also indicated a noticeably lower systemic toxicity of derivative **1a***EE* than cisplatin. All these results indicate that complex **1a***EE* is a suitable leading candidate for further development [3]. The influence of different substituent groups in the benzyl moiety on the biological activity of the iminoether complexes *cis*- and *trans*-$[PtCl_2\{EE\text{-}N(H)=C(OMe)CH_2\text{-}C_6H_4\text{-}p\text{-}R\}]$ (R = CH_3, OCH_3, F) was also investigated. It was observed that *cis*-isomers were significant more potent than the corresponding *trans*-species against all tumor cell lines tested; moreover the complexes bearing CH_3 and F sustituents showed IC_{50} values from about two-fold to six-fold lower than those exhibited by cisplatin [4]. The

reversion of structure-activity relationships of antitumor platinum complexes was observed moving from iminoether ligands in complexes of the type $[PtCl_2\{E\text{-}N(H)=C(OMe)CH_2Ph\}_2]$ exhibiting a higher cytotoxicity in the *cis* form to amidine ligands in complexes of the type $[PtCl_2\{N(H)=C(NR'R'')R\}_2]$ ($R=CH_3$, $R'=H$, $R''=CH_3$; $R'=R''=CH_3$; $R=Ph$, $R'=H$, $R''=CH_3$; $R'=R''=CH_3$) more active in the *trans* form. The new amidine platinum(II) complexes were tested for their *in vitro* cytotoxicity on a panel of various human cancer cell lines. The results indicated that the benzamidine complex *trans*-$[PtCl_2\{N(H)=C(NMe_2)Ph\}_2]$ was the most effective derivative also circumventing acquired cisplatin resistance as demonstrated by chemosensitivity tests performed on cisplatin sensitive and resistant cell lines [5].

References

[1] L.R. Kelland and N.P. Farrell (eds), Platinum based drugs in cancer therapy, Humana, Totowa, 2000.
[2] M. Coluccia and G. Natile, Anti-Cancer in Medicinal Chemistry, 7 (2007) 111
[3] S. Mazzega Sbovata, F. Bettio, M. Mozzon, R. Bertani, A. Venzo, F. Benetollo, R. A. Michelin, V. Gandin, C. Marzano, J. Med. Chem. (2007), accepted
[4] S. Mazzega Sbovata, F. Bettio, C. Marzano, et al, J.Inorg. Biochem. submitted
[5] C. Marzano, S. Mazzega Sbovata, F. Bettio, R.A. Michelin, et al.J. Biol. Inorg. Chem., 12 (2007) 477.

O.12

Antiproliferative effects of prolindac, a novel dach-platinum linked polymer-compound, as a single agent and in combination with other anticancer drugs in a panel of human cancer cell lines

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ProLindac (AP5346) comprises a novel hydrophilic biocompatible copolymer to which a bioactive DACH-platinum (Pt) is attached. ProLindac has recently entered clinical trials. The pH-dependent polymer delivery system is intended to exploit the "leaky" vasculature phenomenon, increasing drug delivery to the tumor site. Here, we determine the optimal pharmacological conditions for ProLindac cytotoxicity, as single-agent or in combination with other cytotoxics, and benchmark ProLindac against oxaliplatin and cisplatin. Antiproliferative effects were evaluated in a panel of human cancer cell lines by MTT assay. Apoptosis induction was evaluated by FACS analysis. Pt levels were measured by atomic absorption. Gene expression was determined using q-RT-PCR. The effects of combining ProLindac with 5-FU, docetaxel, gemcitabine and SN-38 were determined by median effect plot analysis. ProLindac displayed concentration- and time-dependent cytotoxic effects against a broad range of human cancer cell lines (IC_{50}: 3-85 µM), HT29 being the most sensitive; prolonged exposure (72 hours) was the most effective schedule. The expression of several genes of DNA repair systems and drug metabolism (MLH1, MDR1, GSTP1) correlated with ProLindac cytotoxicity. At equiplatinum concentrations, ProLindac and oxaliplatin displayed similar spectra of activity suggesting similar molecular mechanisms of action, that differed from that of cisplatin. Exposure to 120 µM ProLindac (300 ng/ml Pt) led to incorporation of approximately 0.1 µg Pt per mg of DNA. Similar to that of oxaliplatin, ProLindac seems to induce p21 expression and 48-hour exposure to IC_{50} concentrations of ProLindac led to the accumulation of cells in the G2/M phase of cell cycle and apoptosis induction in p53-mutated HT29 and wild-type p53 HCT116. When combined with other cytotoxics such as 5-FU, gemcitabine, and SN-38, ProLindac showed combination index values <1, suggesting synergistic effects. Administering ProLindac for 48 hours, after gemcitabine and 5-FU, resulted in higher cytotoxicity than other sequences. For SN-38, no schedule-dependency was found. Results for the combination of ProLindac/oxaliplatin and docetaxel varied between cell lines; in HCT116, HT29 and OVCAR3 cells, an additive effect was seen, independently of administration schedule. In IGROV1 cells, administering docetaxel prior to platinum showed synergistic effects. The effects of several schedules of administration were similar to those of oxaliplatin combinations in colon and ovarian cancer cell lines. ProLindac displayed a cytotoxic profile and similar molecular and cellular effects to that of oxaliplatin, alone and in combination with other cytotoxics. Based on these findings, the biopolymer-linked DACH-Pt, ProLindac, merits further study in clinical trials.

O.13

Comparison of innovative metal-based drugs on the metastatic progression of breast cancer cells

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Distant metastases are life-threatening events in the course of most solid tumours and represent the real target of chemotherapy. To provide a significant clinical advancement, new concepts should drive the design of new drugs, in order to get selectivity. Among metal compounds HIm[Ru(III)Cl_4Imdmso] (NAMI-A), has emerged for its ability to selectively interfere with metastasis formation and growth, with marginal inhibition of primary tumours. The aim of this study is to evaluate the capacity of some representative heterocyclic (HIm[Ru(III)Cl_4Im_2], KP418; HInd[Ru(III)Cl_4Ind_2], KP1019) and organometallic (Ru(II)$Cl_2(\eta^6\text{-}C_7H_8)$(PTA), RAPTA-T; [($\eta^6$-byphenyl)Ru(II)Cl(en)]$PF_6$, RM175; [($\eta^6$-byphenyl)Os(II)Cl(en)]$BF_4$, AFAP51) complexes to interfere with some steps of the metastatic progression, mimed by appropriate *in vitro* tests, and to compare these results with their *in vivo* anti-metastatic activity. For this purpose tests have been carried out that simulate the detachment from the primary tumour, the degradation of the extracellular matrix, the ability to migrate, to invade and to adhere to a new organ, comparing the behaviour of MDA-MB-231 highly invasive breast cancer cells to that of HBL-100 non tumorigenic mammary epithelial cells. Evaluation of anti-metastatic activity has been studied on the MCa mammary carcinoma of the CBA mouse. *In vitro* experiments highlight the inability of KP1019 and RM175 to distinguish between tumour and no-tumour cells. KP1019 shows pronounced inhibition of cell motility and invasion as well as of gelatinolytic activity, however all these effects are influenced by its cytotoxicity. On the contrary RAPTA-T, in all tests, shows selectivity for the invasive cell line, with marginal or no effect on the non-tumourigenic one. KP418 and AFAP51 have, generally, weak effects. *In vivo* both RM175 and KP1019 suppress tumour metastasis, an effect attributable to the inhibition of primary tumour growth. On the contrary the effect of RAPTA-T on primary tumour growth is virtually negligible, but metastasis weight drops to approximately 30% of controls. KP418 and AFAP51 appear the least active also in the *in vivo* experiment. These data stress the importance of evaluating the processes of migration, of invasion and of the adhesion ability of treated cells, events that can predict the selective anti-metastatic properties of metal compounds. Moreover by tuning the ligands of the metal centre it is possible to increase the selectivity for tumour metastases leading to ruthenium compounds with strong anti-metastatic activity.

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O.14

CTR1 as a determinant of platinum drug transport

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The human copper transporter 1 (hCTR1), the major transporter responsible for Cu influx, mediates one component of the cellular accumulation of cisplatin (DDP), carboplatin (CBDCA) and oxaliplatin (L-OHP). Studies conducted in wild type murine embryonic fibroblasts ($CTR1^{+/+}$) and a subline in which both alleles of CTR1 were deleted ($CTR1^{-/-}$) demonstrated that, when exposed to 2 μM DDP accumulation in the $CTR1^{-/-}$ cells at 1 h was only 35 – 36% of that in the $CTR1^{+/+}$ cells. Similar results were obtained withy CBDCA and L-OHP. Uptake after just a 5 min exposure to DDP was even more markedly reduced. There was an association between the effect of loss of CTR1 function on uptake of the Pt drugs and their cytotoxicity. The $CTR1^{-/-}$ cells were 3.2-fold resistant to DDP, 2.0-fold resistant to CBDCA but only 1.7-fold resistant to L-OHP. Thus, while CTR1 controls the cellular accumulation of all 3 drugs at low concentrations, accumulation of L-OHP is not as dependent on CTR1 at higher concentrations. CTR1 is rapidly down-regulated by both Cu and DDP in murine embryo fibroblasts and human ovarian carcinoma cells. The mechanism of this effect was investigated using digital deconvolution microscopy and western blot analysis of cells stained with antibodies directed at both ends of the protein. Treatment of human ovarian 2008 cells with DDP in combination with inhibitors of various endosomal pathways (amiloride, cytochalasin D, nystatin and methyl-β-cyclodextrin) demonstrated that hCTR1 degradation was blocked by amiloride and cytochalasin D indicating that hCTR1 was internalized primarily by macropinocytosis. Expression of transdominant negative forms of dynamin I and Rac demonstrated that loss of hCTR1 was not dependent on pathways regulated by either of these proteins. DDP-induced loss of hCTR1 was blocked by the proteosome inhibitors lactacystin, proteosome inhibitor 1 and MG132. The results indicate that DDP-induced down-regulation of hCTR1 involves internalization from the plasma membrane by macropinocytosis followed by proteasomal degradation. Down-regulation of CTR1 also requires the function of the copper chaperone ATOX1. Neither Cu nor DDP caused down-regulation of CTR1 in mouse embryo fibroblasts in which both alleles of *ATOX1* had been knocked out. Since hCTR1 is a major determinant of early DDP uptake, prevention of its degradation offers an approach to enhancing tumor sensitivity. Inhibition of proteosomal function in 2008 cells with bortezomib blocked the DDP-induced down-regulation of CTR1 as determined by western blotting, confocal microscopy and flow cytometry. Exposure to bortezomib also increased DDP accumulation and increased sensitivity to the cytotoxic effect of DDP. We conclude that: 1) CTR1 mediates the initial influx of the Pt drugs when they are present at low, clinically relevant, concentrations; 2) that CTR1-mediated uptake of DDP requires endocytosis; and, 3) that inhibition of the degradation of CTR1 can enhance DDP uptake and cell killing. It remains to be determined whether CTR1 is an important determinant of the *in vivo* activity of DDP.

O.15

ATP7B regulates the export of platinum drugs

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The discovery that the brain- and liver-specific Cu exporter ATP7B, and its ubiquitously expressed homolog ATP7A, mediate resistance to cisplatin (DDP), carboplatin and oxaliplatin and the finding that ATP7A and ATP7B are expressed in aggressive and Pt-drug resistant tumours has identified these P-type ATPases as important targets for drug development and design of predictive strategies. ATP7A and ATP7B are located in the post Golgi secretory vesicles and contain six cytosolic metal binding motifs that bind Cu and trigger the hydrolysis of ATP and the eventual intravesicular sequestration of Cu. Analysis of human 2008 ovarian carcinoma cells and mouse fibroblasts has suggested that ATP7A and ATP7B act as direct transporters of DDP as evidenced by the observation that over expression of these transporters increases accumulation of DDP in the vesicular compartment, and in the case of ATP7B, enhances the efflux rate of DDP. Digital fluorescent microscopy, Western blotting and transport assays extended these findings to suggest that the ATP7B mediates the export of DDP at least in part via exosomal export of DDP. *In vitro* transport assays involving isolated vesicles from Sf9 cells expressing a wild type human ATP7B showed that ATP7B can function as a direct transporter of DDP by binding the drug and sequestering it into vesicles utilizing ATP. Vesicles engineered to express wild type ATP7B accumulated 108.4 ± 9.1 (SEM) pmol DDP/mg protein when exposed to 2 μM DDP for 10 min. This was 2.0 ± 0.02 (SEM) -fold higher than the 54.2 ± 3.8 (SEM) pmol DDP/mg protein accumulated by the control Sf9 vesicles. The ability of ATP7B to transport DDP was further examined using vesicle engineered to express a transport-defective mutant of ATP7B in which the CPC motif in the sixth transmembrane domain was converted to CPA. Sf9 vesicles expressing the CPA mutant did not transport of DDP. In the absence of ATP, the level of DDP associated with CPA expressing vesicles was not significantly different from that associated with the vesicles expressing the wild type ATP7B but was 1.8 ± 0.01 (SEM)-fold higher than that accumulated by the control Sf9 vesicles. The presence of 5 mM ATP increased the levels of DDP transported by nearly 30% but only in the vesicles that expressed the wild type ATP7B. The ATP-dependent accumulation of DDP in ATP7B-expressing vesicles was pH dependent and the highest level of DDP accumulation was detected at pH 4.6 with an apparent K_m of 1.2 ± 0.5 (SEM) μM and a V_{max} of 30.0 ± 2.0 (SEM) μmol/mg protein/min. Compared to Cu, the ATP-dependent transport of DDP was much smaller in magnitude as we recorded a K_m of 3.4 ± 0.4 (SEM) μM and a V_{max} of 800.0 ± 50.0 μmol/mg protein/min for Cu. Similar to Cu, but to a much smaller extent, DDP induced the transient formation of an acylphosphate intermediate ATP7B, confirming that ATP hydrolysis is required for the intravesicular sequestration of DDP. Cu and DDP inhibited the ATP-dependent transport of each other in a noncompetitive manner, suggesting that modulation of intracellular levels of Cu may play a role in altering the efficacy of DDP in tumor cells. These results establish that DDP is a substrate for ATP7B, but is transported at a much slower rate than Cu.

O.16

Molecular alterations of human tumor cells resistant to oxaliplatin

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Platinum drugs are employed in a wide range of solid tumors, and represent the mainstay of the first-line therapy of ovarian carcinoma. Although cisplatin has shown efficacy in the treatment of different types of tumors including ovarian carcinoma, resistance to treatment is a major limitation. In an attempt of overcoming resistance mechanisms a large effort has been made over the years to generate compounds with different geometry. At present, one of the most clinically relevant cisplatin analogue is the mononuclear compound oxaliplatin, which has shown activity and a favourable pharmacological profile in epithelial ovarian cancer. In cellular models, oxaliplatin exhibits activity in some cisplatin-resistant cell lines with acquired resistance to cisplatin, whereas in other models cross-resistance with cisplatin is observed. In general, oxaliplatin and cisplatin exhibit different sensitivity profiles indicating differences in drug-DNA interaction and/or cellular response or detoxification. Thus, it is likely that differences in the influx or efflux mechanisms for these drugs also contribute to their unique patterns of clinical activity and can at least in part contribute to resistance. Impaired drug accumulation has been recognized over the years as a frequent feature of cells resistant to cisplatin and more recently as an alteration of oxaliplatin-resistant models. Based on the clinical interest of oxaliplatin, we have developed an oxaliplatin-resistant subline of the human ovarian carcinoma cell line IGROV-1 (IGROV-1/OHP) by in vitro continuous exposure of parental cells to oxaliplatin, and drug-resistant clones by pulse exposure. IGROV-1/OHP resistant cells exhibited a high degree of resistance to the selecting agent, cross-resistance to cisplatin and to topotecan. In such cells, resistance to platinum drugs was associated with multiple alterations including p53 mutation and deregulation of survival pathways. A marked decrease of drug accumulation was observed in IGROV-1/OHP cells as compared to parental cells after exposure to oxaliplatin and cisplatin. Such a feature was not related to down-regulation of the expression of the human copper transporter 1. Resistant clones, which carried-wild type p53, exhibited low levels of resistance to oxaliplatin, that was associated with marginal changes in drug accumulation. Our results indicate that multiple alterations are associated with development of resistance to oxaliplatin as defects in drug accumulation and alterations in cell response to damage were found. The present study supports the interest of the established models in the development of therapeutic strategies aimed at specific cellular targets.

O.17

How to overcome cisplatin resistance through proton pump inhibitors

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Tumor microenvironment plays a key role in tumor malignancy. In fact, recent data support a key contribution of hypoxia and acidity in the progression from benign to malignant growth. In particular, the unfavourable environment may induce the selection of tumor cells able to survive in acidic and hypoxic conditions. The common components of the cancer phenotype result from active selection, and characteristics of tumor microenvironment may create the best condition for this selection. Acidity, in particular, has been shown to have a role in resistance to chemotherapy, proliferation and metastatic behaviour. In fact, a mechanism of resistance to cytotoxic drugs may be the alteration of the tumor microenvironment through changes of the pH gradient between the extracellular environment and cell cytoplasm. The extracellular pH of solid tumors is significantly more acidic than that of normal tissues, thus impairing the uptake of weakly basic chemotherapeutic drugs and reducing their effect on tumors. Malignant tumor cells seem to hijack some of these mechanism to protect themselves from the acidic environment and to maintain acidity in an environment unsuitable for normal or more differentiated cells. Recent data suggest that vacuolar-type (V-type) H+-ATPases represent one of the major mechanism in tumor resistance to drugs, through acidification of extracellular and intracellular compartments. In fact,. H+-ATPases pump protons across the plasma membrane, and widely expressed in normal and tumor issues. Some human tumor cells are characterized by an increased V-type H+-ATPase expression and activity, and pretreatment with proton pump inhibitors – a class of H+-ATPase inhibitors – sensitized tumor cell lines to the effect of a variety of anticancer drugs. Proton pump inhibitor pretreatment has been associated with inhibition of V-type H+-ATPase activity and increase in both extracellular pH and pH of lysosomal organelles. Between the drugs that suffer tumor resistance is cisplatino, that particularly in the treatment of solid tumor has proven to be ineffective. This study was aimed at investigating whether proton pump inhibitors (PPIs), currently used in the anti-acid treatment of peptic disease, could inhibit the acidification of the tumor microenvironment and increase the sensitivity of tumor cells to cisplatin as compared to other cytotoxic agents. In fact, we knew that PPIs

To this purpose cell lines derived from human melanomas, adenocarcinomas, and lymphomas were pre-treated with the PPIs omeprazole, esomeprazole, or pantoprazole and tested their response to cisplatin or other cytotoxic drugs in cell death assays. We also evaluated extracellular and intracellular pH and vacuolar-H_-ATPase (V-H_-ATPase) expression, distribution, and activity in PPI-pretreated cells by using western blot analyses, immunocytochemistry, laser scanning confocal analysis, and bioluminescence assays. We also preformed some in vivo experiments in a model of SCID mice transplanted s.c. with either melanoma or osteosarcoma cells and then treated in vivo with the various

combination. In these xenograft we evaluated both changes in the pH of human tumors and the tumor growth and cisplatin sensitivity with or without omeprazole pretreatment.
PPI pretreatment sensitized tumor cell lines to the effects of cisplatin, but also of 5-fluorouracil, and vinblastine, with an IC50 value reduction up to 2 logs. PPI pretreatment was associated with the inhibition of V-H_-ATPase activity and increases in both extracellular pH and the pH of lysosomal organelles. PPI pretreatment induced a marked increase in the cytoplasmic retention of the cytotoxic drugs, with clear targeting to the nucleus in the case of doxorubicin. In in vivo experiments, we first demonstrated that the pH of human tumors is substantially lower than normal tissues and that PPI treatment induce icrease of tumor pH and marked changes in the various pH gradients. Then we showed that oral pretreatment with omeprazole was able to induce sensitivity of human solid tumors to cisplatin.
Our results open new possibilities for the treatment of drug-resistant tumors through combination strategies based on the use of well-tolerated pH modulators such as PPIs. Two clinical trials are now ongoing in Italy based on the use of PPIs pretreatment as an approach to chemosesibilize the malignant tumors.

O.18

Multiple mechanisms of resistance to cisplatin in cancer cells

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We have studied the development of resistance to cisplatin in cultured KB-3-1 human cervical adenocarcinoma cells and BEL-7404 human hepatoma cells. In a single-step selection, it is possible to develop a pleiotropic phenotype consisting of the following: (1) cross-resistance to other platinum compounds, arsenite, cadmium, methotrexate, and nucleoside analogs; (2) reduced accumulation of multiple platinum compounds, other heavy metals and methotrexate; (3) reduced receptor-mediated and fluid phase endoctytosis; (4) altered cytoskeleton, including disruption of actin microfilaments, filamin structures, and microtubules; (5) a defect in protein trafficking consisting of relocalization to the inside of the cell of several different multi-transmembrane proteins such as the ABCC1 transporter (MRP1, GS-X pump), glucose transporter (Glut1), and folate binding protein (FBP); and (6) altered mitochondrial morphology and function. Additional steps of resistance increase the extent of this pleiotropic phenotype and affect methylation of several genes.
Our hypothesis, based in part on the existing literature, is that cisplatin can enter the cell in many different ways, either by piggybacking on many different transporters, or entering via fluid phase endocytosis. To obtain resistant cells, several of these mechanisms must be simultaneously inactivated, which is precisely what happens in the cell lines we have isolated by stepwise growth in cisplatin. The likely cause of this pleiotropic alteration is constitutive activation of a regulatory switch that results in disruption of membrane protein trafficking, possibly by a primary defect in cytoskeletal organization. Consistent with this hypothesis, we find that RhoA, a regulator of cytoskeletal structure, is reduced in resistant cells. We speculate that this results in altered trafficking of plasma membrane proteins, including those involved in normal uptake of cisplatin into cells, and defective fluid phase endocytosis. One additional consequence of reduced glucose uptake that occurs because of the mislocalization of Glut1 is a mitochondrial metabolic phenotype that reduces apoptotic signals.

O.19

Elevated glutathione levels confer cellular sensitization to cisplatin toxicity by upregulation of copper transporter hCTR1

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Previous studies have demonstrated that treating cultured cells with cisplatin (CDDP) upregulated the expression of glutathione (GSH) and its de novo rate-limiting enzyme, gamma-glutamylcysteine synthetase (r-GCS), which consists of heavy (r-GCSh) and light (r-GCSl) subunits. It has also been shown that many CDDP-resistant cell lines exhibit high levels of r-GCSh/r-GCSl and GSH. Since GSH system is the major intracellular regulator of redox conditions that serve as an important detoxification cytoprotector, these results have been taken into considerations that elevated levels of r-GCS/GSH are responsible for the CDDP resistance. In contrast to this context, we demonstrated here that overexpression of GSH by transfection with expression recombinant plasmid containing the r-GCSh cDNA conferred sensitization to CDDP through upregulation of human copper transporter 1 (hCtr1), which is also known as transporter for CDDP. Depleting GSH levels in these transfected cells reversed CDDP sensitivity with concomitant reduction of hCtr1 expression. While expression of Cu transport was also upregulated in the transfected cells, these cells exhibited biochemical signature of Cu deficiency, suggesting that GSH functions as an intracellular Cu-chelator and that overexpression of GSH can alter Cu physiology. More importantly, our results redefine a new role of GSH in the regulation of CDDP sensitivity. Overproduction of GSH depleted bioavailable Cu pool, leading to upregulation of hCtr1 and sensitization of CDDP transport and cell killing. These findings also have important implications that modulation of intracellular Cu pool may be a novel strategy for improving chemotherapeutic efficacy of platinum-based antitumor agents.

O.20

Mechanism of cellular uptake and cytotoxicity of cisplatin nanocapsules

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Cisplatin is one of the most widely used agents in the treatment of solid tumors. However, its clinical use is limited by toxic side effects. We have developed a novel method for the efficient encapsulation of cisplatin in a lipid formulation that may reduce the side effects and increase the therapeutic efficacy of cisplatin in the clinic (Burger et al., Nat Med 8, 2002). The method is unique in that it generates nanocapsules, nanoprecipitates of cisplatin covered by a single lipid bilayer. The nanocapsules have an in vitro cytotoxicity toward IGROV1 human ovarian carcinoma cells up to 100-fold higher than the free drug. By direct measurement of cellular platinum-accumulation we have demonstrated that the increased cytotoxicity of cisplatin nanocapsules results from a much more efficient uptake of encapsulated cisplatin in the cells compared to the free probe. This enhanced accumulation was shown to lead to increased formation of DNA-adducts that ultimately results in cell death.
To investigate the origin of the increased accumulation of cisplatin in the cells after treatment with cisplatin nanocapsules, the interaction of fluorescently labeled cisplatin nanocapsules with tumor cells was investigated by confocal fluorescence microscopy. The results from these experiments confirm that fluorescent cisplatin encapsulated in nanocapsules is taken up much more efficiently than the free probe. Also, the fluorescently labeled lipid is taken up by the cells, indicating that the nanocapsules are taken up via endocytosis. Using inhibitors, co-staining with organelle-specific antibodies, and shRNA oligo's to downregulate clathrin heavy chain and caveolin1, the role of endocytosis in the uptake process is currently further addressed.

O.21

Altered localization of transport proteins associated with cisplatin resistance in human ovarian carcinoma cells

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Despite the success of cisplatin-based anticancer chemotherapy, its clinical application is limited because tumors often develop resistance after repeated drug administrations. Acquired resistance to cisplatin has been intensively studied. However, the mechanisms that mediate cellular uptake and efflux of cisplatin have not yet been fully elucidated. Recent findings suggest the involvement of copper homeostasis proteins in the intracellular transport of the drug.[1]
In the present study, intracellular localization of the copper efflux transporters, ATP7A and ATP7B, in the A2780 human ovarian carcinoma cell line and its cisplatin-resistant variant A2780cis cell line was investigated. In sensitive A2780 cells, both ATP7A and ATP7B localize to the perinuclear region (presumably trans-Golgi network). In cisplatin-resistant cells, ATP7A and ATP7B are redistributed away from the trans-Golgi to more peripherally located vesicles in the cytosol. Treatment of A2780 cells with cisplatin triggered reversible relocalization of both proteins from the perinuclear region to the above-mentioned peripheral sites in the cytoplasm. Colocalization experiments using a cisplatin analogue labeled with carboxyfluoresceindiacetate showed that both ATP7A and ATP7B mediate cisplatin transport in A2780 cells, but only ATP7A (and not ATP7B) colocalizes with a fluorescent cisplatin analogue in resistant A2780cis cells.
Thus, in spite of altered locatization in the A2780cis cell line, ATP7A mediates either cisplatin efflux or its sequestration. Sequestration is more likely to take place given the enhanced expression of the protein in the resistant cell line and nonetheless similar efflux rate in the A2780 and A2780cis cells. It is also in agreement with previous findings suggesting that ATP7A regulates cisplatin sensitivity by sequestering it in vesicular structures.
In contrast, relocalization of ATP7B away from the trans-Golgi network in A2780cis cells prevents cisplatin binding to this protein. As a result, ATP7B may not be able to perform cisplatin efflux and the drug may be sequestrated inside the cell. In this way, altered localization of ATP7B (and not the expression of this protein, which is similar in the sensitive and resistant cell lines) appears to contribute to cisplatin resistance in A2780cis cells.
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References
1. Safaei, R.; Howell, S. B. Crit. Rev. Oncol. Hematol. 2005, 53, 13.

O.22

New metastable cationic complexes of the type $[PtCl(\eta^2\text{-}C_2H_4)(N\text{-}N)]^+$ as possible antitumor *pro*-drugs

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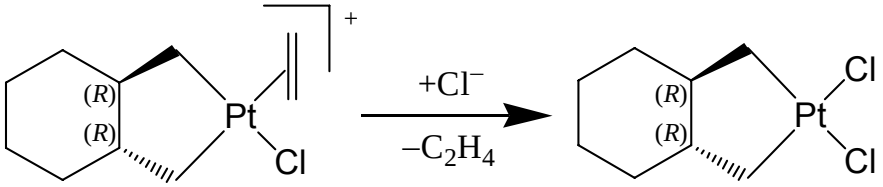
Cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, is the prototype of an increasing family of platinum-based drugs having formula *cis*-$[PtA_2X_2]$ (A_2 = two amines or a diamine, X_2 = two monoanions or a dianion). Its activity is attributed to the formation of DNA adducts involving two purines crosslinked at the *N7* atoms.[1-3] Oxaliplatin, [*R,R*-1,2-diaminocyclohexane(oxalato-*O,O'*)platinum(II)], is a third-generation platinum compound that has shown a wider spectrum of activity than cisplatin and the lack of cross-resistance with cisplatin and carboplatin. Oxaliplatin contains the non-leaving chiral *R,R*-1,2-diaminocyclohexane ligand, *R,R*-**DACH**, and an oxalate dianionic ligand which can undergo solvolysis.[4,5] Cellular deficiency in mismatch repair (MMR) and increase in replicative bypass, which appear to contribute to cisplatin resistance, do not appear to induce a similar resistance to oxaliplatin.[6]

In this work two new Pt(II) enantiomeric cationic complexes $[PtCl(\eta^2\text{-}C_2H_4)$(*R,R*-**DACH**)]Cl, **1**, and $[PtCl(\eta^2\text{-}C_2H_4)$(*S,S*-**DACH**)]Cl, **2**, (**DACH** = *trans*-1,2-diaminocyclohexane) were synthesized. Such complexes are very water soluble in the chloride form and show an unusual kinetic stability toward the olefin loss, see Scheme. NMR monitoring demonstrated that in the presence of excess chloride both **1** and **2** slowly ($t_{1/2} \approx 48$ h at 25°C and $[Cl^-]$ = 1M) undergo olefin loss, with formation of the neutral dichloro species $[PtCl_2$(*R,R*-**DACH**)], **3**, and $[PtCl_2$(*R,R*-**DACH**)], **4**, respectively. The positive charge that increases water solubility of **1** and **2** it is expected, in principle, to limit cellular uptake. On the other side for the neutral and low soluble **3** and **4** it is expected a strong cell membrane uptake.

Cytotoxicity on HeLa cancer cells evaluated by MTT tests showed for **1** and **2** a lower but interesting cytotoxic activity with respect to the clinically used antitumor drugs cisplatin, *cis*-diamminedichloroplatinum(II) and oxaliplatin. Interestingly compound **1** which carries the same diamine ligand of oxaliplatin (*R,R*-**DACH**) showed higher cytotoxicity on HeLa cells with respect to its enantiomer. Time course experiments showed that cytotoxicity of **1** strongly increases with time, with respect to **2**, corresponding to formation of the dichloro complex **3**. Since the decomposition of soluble **1** it is expected to give products similar to those obtained in the decomposition pathway of the active antitumor drug oxaliplatin, this suggested us a possible use of **1** as antitumor *pro*-drug.



References
[1] D. Wang, S. J. Lippard *Nat. Rev. Drug Discovery* 2005, *4*, 307.
[2] J. Reedijk *Proc. Natl. Acad. Sci. U.S.A.*. 2003, *100*, 3611.
[3] E. Wong, C. M. Giandomenico *Chem. Rev.* 1999, *99*, 2451.
[4] M. Benedetti, J. Malina, J. Kasparkova, V. Brabec, G. Natile *Environ. Health Perspect.* 2002, *110*, 779.
[5] A. M. Di Francesco, A. Ruggiero, R. Riccardi, *Cell Mol. Life Sci.* 2002, *59*, 1914.

O.23

Structural and mechanistic studies of anticancer platinum drugs: uptake, activation, and the cellular response to DNA binding

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There are three stages by which platinum anticancer drugs exhibit their antitumor activity: cell entry, drug activation, and Pt–DNA binding with its attendant consequences. Our research, discussed in this presentation, has led to the following results. (1) The effectiveness of oxaliplatin is a consequence of its selective delivery to cells containing organic cation transporters hOCT1 and hOCT2. (2) Intrastrand cisplatin 1,3-GTG cross-links determine and override the natural positioning of DNA on the nucleosome core particle; intrastrand 1,2-GG cross-links on nucleosomes have somewhat different structures. (3) The X-ray structure of a site-specific monofunctional platinum-DNA duplex reveals profound differences in structure from DNA bearing a 1,2- or 1,3-intrastrand cross-link. (4) Both monofunctional and bifunctional platinum-DNA adducts are strong transcription blockers and are removed to differing degrees by the nucleotide excision repair pathway. (5) The potency of platinum drugs depends on a competition between transcription inhibition and repair. (6) Photo-cross-linking of platinated DNA to cellular proteins reveals several factors that may process the platinum adducts. (7) New cell-targeting modalities, including the tethering of cellular receptor moieties and the use of single-walled carbon nanotubes as a "longboat delivery system" offer novel ways to carry the potent platinum warhead to cancer cells for both mechanistic and anticancer drug development work. Our studies are supported by the US National Cancer Institute.

O.24

Molecular rationale to improve platinum selectivity

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Abstract not received

O.25

Differences in conformation and conformational dynamics between cisplatin and oxaliplatin DNA adducts may explain the selective recognition of those adducts by cellular proteins

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Some DNA damage-recognition proteins, transcription factors, mismatch repair proteins and DNA polymerases discriminate between cisplatin (CP)- and oxaliplatin (OX)-GG DNA adducts, and this is thought to help explain differences in efficacy, toxicity and mutagenicity of CP and OX. In addition, differential recognition of CP- and OX-GG adducts by some proteins has been shown to be highly dependent on the sequence context of the Pt-GG adduct. We have postulated that CP- and OX-GG adducts cause differences in the conformation and/or conformational dynamics of the DNA that provide the basis for differential protein recognition of the adducts. We have determined the NMR solution structure of CP-GG adducts, OX-GG adducts and undamaged DNA in the AGGC sequence context, and of OX-GG adducts and undamaged DNA in the TGGT sequence context. These data show that CP-GG adducts differ from OX-GG adducts for several DNA helical parameters in both AGGC and TGGT sequence contexts. In addition, OX-GG adducts in the AGGC sequence context differ from OX-GG adducts in the TGGT sequence context. We have also employed molecular dynamics (MD) simulations to investigate the conformational dynamics of CP-GG adducts, OX-GG adducts and undamaged DNA in the AGGC, TGGA and TGGT sequence contexts. These studies showed clear differences in the conformation dynamics between CP- and OX-GG adducts which correlated with the average conformational differences observed in the NMR solution structures and with conformations previously reported for the CP-GG DNA•HMG1a complex. When the conformational dynamics in all three sequence contexts were compared it became evident that: 1) the patterns of hydrogen bond formation between Pt-amine-hydrogens and surrounding bases of the DNA were different for CP- and OX-GG adducts and 2) the differences in patterns of hydrogen bond formation were highly correlated with differences in the conformational dynamics of the adduct. Thus, we postulate that patterns of hydrogen bond formation between Pt-amine hydrogens and surrounding DNA bases are different for CP- and OX-GG adducts, and that those differences in hydrogen bond patterns result in DNA conformational differences that allow selective recognition of CP- and OX-GG adducts by a number of proteins that determine the relative cytotoxicity and mutagenicity of those adducts.

O.26

Platinum drugs and DNA repair. Lessons from the NCI-60 panel and clinical correlates

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The cellular processing of platinum drugs involves numerous events, which may play a role in their efficiency: uptake and efflux, formation, recognition and repair of DNA adducts, transduction of DNA damage signals, induction of cell death. The expression and/or intrinsic activity of the proteins involved may determine the level of sensitivity of tumour cells. A model for studying the relative importance of these events is the NCI-60 panel, consisting of a panel of 60 human tumour cell lines that have been characterised in terms of drug sensitivity and of molecular targets such as gene expression profiles and polymorphisms. Data mining may lead to significant observations, providing tracks for the clinical evaluation of potential predictors of drug efficacy. We have already shown that the molecular determinants of the in vitro cytotoxicity of cisplatin and oxaliplatin were considerably different, suggesting the existence of a special mechanism of action for oxaliplatin.
We have explored some pathways involved in recognition and repair of platinum drug-induced DNA damage, using the database of the NCI as well as personal results obtained on this model. We show that the expression of HMGB1, which targets platinum-DNA adducts and prevents DNA repair, is positively correlated with the cytotoxicity of all the platinum drugs tested. Among the proteins involved in NER, ERCC1 expression is positively correlated with the cytotoxicity of cisplatin and carboplatin, but not of the DACH platinum drugs, oxaliplatin and tetraplatin, while ERCC2 expression is negatively correlated with the cytotoxicity of oxaliplatin and tetraplatin, but not of cisplatin or carboplatin. The expression of the major proteins involved in MMR, MLH1 and MSH2, is strongly correlated with the cytotoxicity of platinum drugs, in relation to the inefficient mobilisation of these proteins to repair platinum-DNA adducts. The SNP asn118asn of ERCC1 appears involved in the cytotoxicity of cisplatin and carboplatin, the variant cell lines being significantly less sensitive to these drugs than the wild-type cell lines or the heterozygous ones. The SNPs asp312asn and lys751gln of ERCC2 and asp1104his of ERCC5 did not appear to play a major role in platinum drug cytotoxicity against the NCI-60 panel.
Clinical studies have shown several genes involved in NER played a role in patients' drug response and survival: ERCC1 expression is associated with cisplatin resistance in lung cancer, the ERCC1 asn118asn SNP to oxaliplatin efficacy in colorectal cancer and to cisplatin resistance in lung cancer, the ERCC2 lys751gln SNP to the survival of colorectal cancer patients treated with oxaliplatin. In order to achieve better individualisation of platinum drugs prescriptions, using the tracks provided by the NCI-60 panel could select the appropriate genes to be explored, at the level of either gene expression or polymorphisms. For instance, the exploration of HMGB1 expression as a predictor of drug response is warranted.

O.27

Effects of platinated compounds in signal transduction: role of interaction with other drugs

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Platinum analogs act by formation of DNA adducts, but may also, as the parent compound, interfere with several metabolic processes. Both processes contribute to the final cytotoxicity of this class of agents. The various analogs which are currently being used and being developed for clinical application, have distinct differences in their pharmacokinetic behaviour, their bioavalability, their efficiency to form adducts, the repair processes, and possibly their interference with cellular signalling pathways. The latter may be an underestimated mechanism of action of platinated compounds. Platinum analogs may affect signalling pathways indirectly after formation of DNA adducts, or directly as the compound itself or through a metabolite. The first process is the best characterized process since the formation of DNA adducts will trigger the defence mechanism of the cell to repair the adducts or force the cell into apoptosis. In general the cell death induction can involve both the mitochondrial or receptor pathway. In H460 non-small cell lung cancer (NSCLC) cells activation of caspase-8 was not associated with the death receptor activation inasmuch the Fas-associated death domain. Stable espression of Bcl-2 and bcl-xl suppressed apoptosis and mitochondria-mediated cytochrome c release in cisplatin-treated cells. Since inhibition of caspase 9 did not prevent cisplatin-mediated apoptosis, it was concluded that caspase-8 activates the apical and mitochondrial step leading to apoptosis. Inhibition of the PI3K pathway by LY294002 did not affect oxaliplatin cytotoxicity, indicating that downstream pathways via Akt signalling such as caspase-9 and FasL are possibly not involved in oxaliplatin cytotoxicity as well. Similarly inhibition of the MEK/ERK pathway by PD98059 did not affect oxaliplatin cytotoxicity. However, inhibition of the p38-MAPK pathway by SB203580 protected cells partially against oxaliplatin cytotoxicity. Other signalling pathways involved in the mechanism action of platinum compounds are directly related to formation and repair process of DNA adducts. Both processes involved the activation and inactivation of several protein kinases. However, such an interaction may be cell line and platinum analog dependent. E.g. DNA adduct formation induced by either oxaliplatin and satraplatin (as JM118) is not repaired by the mismatch repair system, hence this system will not trigger signalling pathways as might the case for cisplatin. However, JM118 may trigger the Akt pathway as was seen in the relative insensitive A549 NSCLC cells, but inhibited it in the sensitive A2780 ovarian cancer cell lines. In addition p-Erk phosphorylation was induced in A549 cells, but not in A2780 cells. Such effects on signalling pathways form a rational background for combination of platinum analogs with drugs inhibiting these signalling pathways such as erlotinib. Indeed in the sensitive A2780 erlotinib did not enhance the effect of JM118, but it did in the A549 cells.

O.28

Effect of sequence context on conformational dynamics of DNA adducts of platinum anticancer drugs: MD simulations of cisplatin- and oxaliplatin-DNA adducts

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DNA binding proteins, such as HMG-domain proteins, discriminate between Cisplatin (CP)- and Oxaliplatin (OX)-GG DNA adducts to varying extents depending on the bases flanking the 3' and the 5' side of the adduct. There is a higher level of discrimination between CP- and OX-DNA adducts by the HMG-domain proteins in the TGGA sequence context than in the AGGC sequence context. A thorough understanding of such differential binding affinities would lead to better understanding of the efficacy, toxicity and mutagenesis of these platinum anticancer drugs. We have reported that CP- and OX-GG adducts in the AGGC sequence context differ in the pattern of hydrogen bond formation between platinum amine hydrogens and the surrounding DNA bases and that these differences in hydrogen bond (H-bond) patterns correlate with differences in DNA conformational dynamics that may be important for binding of HMG-domain proteins (Sharma et al, J Mol Biol, epub ahead of print). In this study, we performed 5, 10 ns MD simulations for each of the CP-, OX-, and undamaged DNA duplex in the 5'-d(CCTCTGGACTCC)-3' 5'-d(GGAGTCCAGAGG)-3' sequence context. Conformational analysis of the MD trajectories showed significant structural differences between the non-platinated duplex DNA and those of the CP- and OX-DNA adducts, mainly in the central four base pairs (5' d(T5G6G7A8) 3'). The pattern of H-bond formation between Pt-amines and the surrounding bases was different than observed in the AGGC sequence context. While OX primarily formed H-bonds with G7O6 and T17O4, CP formed H-bonds with G7O6 and A8N7. The snapshots from the trajectory were clustered on the basis of the type of H-bond the drugs were forming, again showing association between H-bond formation and DNA conformational dynamics. Conformational analysis of these clusters revealed significant differences in the 3' side of CP and OX structures when compared to structures in which no H-bonds were formed. In summary, we see differences in H-bond patterns between CP- and OX-GG adducts. These H-bond patterns are influenced by sequence context, and they strongly correlate with differences in DNA conformational dynamics, which may influence the ability of damage recognition and DNA repair proteins to discriminate between CP- and OX-adducts. (Supported by NIH grant CA84480)

Type of Hydrogen Bond	CP (in %)	OX (in %)
N31H - G7O6	31.5	54.6
N31H – T17 O4	-	4.2
N31H -A8N7	12.5	-
Both N31H - G7O6 and N31H - T17O4	-	10.5
None	53.9	23.1

O.29

Photo-cross-linking and identification of proteins that bind to platinum-modified dna in cancer cell extracts

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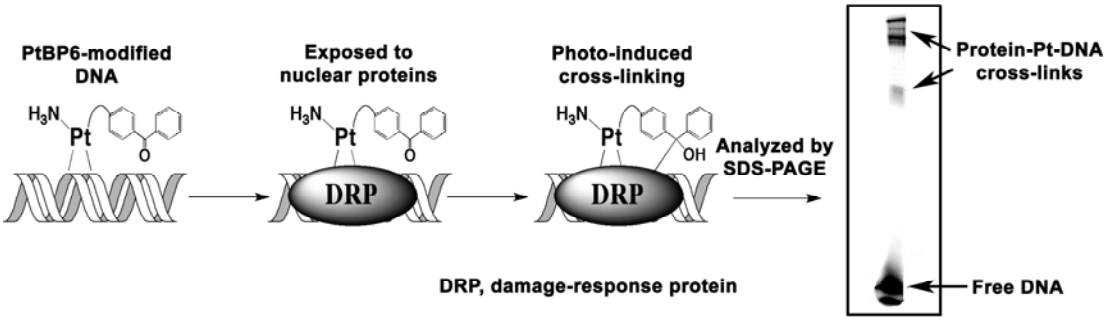
The effects of platinum-DNA adducts in cells have been investigated for many years. The binding of cisplatin to consecutive guanine residues in a DNA strand causes bending and unwinding of the duplex, making the lesions a recognition site for cellular proteins. Photo-cross-linking experiments with the use of a photo-activated analogue of cisplatin (PtBP6) were carried out to isolate and identify proteins that bind to platinum-damaged DNA. The photo-cross-linked proteins depend upon cell type, which may correlate with the sensitivity of the cells to cisplatin treatment. Synthesis and photo-cross-linking of nuclear proteins using a benzophenone-modified DNA probe will also be reported. The involvement of one of the identified proteins, poly(ADP-ribose) polymerase-1 (PARP-1), in cisplatin cytotoxicity was investigated by monitoring PARP-1 activity following cisplatin treatment of various human cancer cell lines. The sensitivity of these cell lines to co-treatment of cisplatin and sub-lethal levels of PARP and PARG inhibitors will be described. This work was supported by a grant from the National Cancer Institute.



O.30

Regrowth resistance: low-level platinum resistance mediated by rapid recovery from platinum-induced cell cycle arrest

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The H69CIS200 and H69OX400 cell lines are novel models of low-level platinum drug resistance developed from H69 human small cell lung cancer cells. The resistant cell lines were developed with eight 4-day treatments of 200 ng/ml cisplatin or 400 ng/ml oxaliplatin respectively, with a recovery period between treatments to emulate the cycles of chemotherapy given in the clinic. The resistant cell lines were approximately 2-fold resistant to cisplatin and oxaliplatin and were cross resistant to both drugs. Platinum resistance was not associated with increased cellular glutathione, decreased accumulation of platinum or increased DNA repair capacity. The H69 platinum sensitive cells entered a lengthy 3 week growth arrest in response to low-level cisplatin or oxaliplatin treatment. This is an example of the coordinated response between the cell cycle and DNA repair. In contrast the H69CIS200 and H69OX400 cells have an alteration in the cell cycle allowing them to rapidly proliferate post drug treatment. The resistant cell lines also have many chromosomal rearrangements most of which are not associated with the resistant phenotype, suggesting an increase in genomic instability in the resistant cell lines. We hypothesised that there was a deregulation between the cell cycle and DNA repair in the resistant cell lines allowing proliferation in the presence of DNA damage which has created an increase in genomic instability. The H69 cells and resistant cell lines have mutant p53 and consequently decrease the expression of p21 in response to platinum drug treatment, promoting progression of the cell cycle instead of increasing p21 to maintain the arrest. An Atlas Toxicology array identified changes in the expression of DNA repair genes, ERCC1 and RAD51B. A decrease in ERCC1 protein expression and an increase in RAD51B foci activity was observed with the platinum induced cell cycle arrest and did not correlate with resistance or altered DNA repair capacity. These changes may in part be mediating and maintaining the cell cycle arrest in place of p21.The rapidly proliferating resistant cells have restored the same levels of both these proteins as untreated cells. Resistance in the H69CIS200 and H69OX400 cells is associated with the speed of the recovery from the cell cycle arrest which may involve modulation of ERCC1 and RAD51B. We use the term ‘regrowth resistance’ to describe this low-level platinum resistance where cells survive treatment through increased proliferation. Regrowth resistance may play a role in the onset of clinical resistance.

O.31

Targeting nucleotide excision repair as a mechanism to increase cisplatin

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The main repair pathway that cells employ to repair cisplatin damage is the nucleotide excision repair (NER) pathway. Recognition of DNA damage is the rate limiting step in this pathway and involves binding of a recognition complex that includes Replication Protein A (RPA) and Xeroderma Pigmentosum Group A (XPA) to the cisplatin-DNA damage site. By targeting and inhibiting recognition, repair of the DNA damage will be reduced and cisplatin adduct levels and duration will be increased. To identify small molecule inhibitors (SMI) of RPA and XPA we developed a high-throughput screening assay using fluorescence polarization (FP). A fluorescent labeled DNA along with purified RPA and XPA were employed in a 384 well format and used to screen the ChemDiv library of chemical compounds. We have screened over 40,000 compounds and identified a series of SMIs for XPA and for RPA. We have analyzed these hits in secondary screens using FP and electrophoretic mobility shift assays, confirming the activity of a subset of both the XPA and RPA hits. Cellular activity of a lead RPA inhibitor was assessed in human lung cancer and ovarian cancer cell line using a series of viability, cell cycle and apoptosis assays. An antiproliferative effects was observed in viability assays and flow cytometric analysis reveal a prolongation of S-phase, consistent with inhibition of RPA's role in DNA replication. Flow cytometry was also employed to measure apoptosis using propidium iodide and annexin V staining. The RPA SMI was found to include apoptosis in both a time and concentrations dependent fashion in both ovarian and lung cancer cell lines. Preliminary analysis in NOD SCID mice reveal minimal toxicity and position us to assess the efficacy of this compound in a lung tumor xenograft model in conjunction with platinum based therapy.

This work was supported by NIH grant CA82741 and funding from the Flight Attendants Medical Research Institute and the Walther Cancer Institute.

O.32

Paraptotic cell death induced by the thioxotriazole copper complex A0: a new tool to kill apoptotic resistant cancer cells

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The copper(II) complex A0 induces non-apoptotic programmed cell death in HT1080 fibrosarcoma cells but not in normal fibroblasts (Tardito S, (2007) J Med Chem, 50(8):1916-24). While typical apoptotic features, such as caspase-3 activation or nuclear fragmentation, are evident in cisplatin-treated cells, they are absent in A0-dependent cell death. In contrast, the latter process is hallmarked by the development of huge vacuoles originating from endoplasmic reticulum (Tardito S, (2006) Histochem Cell Biol 126(4):473-82), a feature consistent with Paraptosis, a newly described alternative cell death type (Sperandio S., (2000) PNAS 97(26):14376-81). Consistently, in a panel of human cancer cells there is no correlation between the sensitivities to A0 and cisplatin. In the same panel, paraptosis-like cell death is observed in all the A0-sensitive cell lines. Moreover, the copper complex kills cisplatin sensitive cells (HT1080 and ovarian carcinoma 2008) and their cisplatin-resistant counterparts (the newly established HT1080PTR and C13*cells) with comparable potency. The different activity spectrum between A0 and cisplatin suggests different mechanisms of action (MOA) for the two drugs. In agreement with this hypothesis, a whole-genome expression analysis, performed in HT1080 cells, showed that the transcriptional response evocated by the two drugs is poorly overlapping. A0 induces genes involved in oxidative- and endoplasmic reticulum-stress (ER stress) while cisplatin increases the expression of typical p53 target genes. Moreover, A0 strongly induces metal responsive genes, like metallothioneins and metal transporters, as well as HSPs, chaperones and other genes involved in the Unfolded Protein Response (UPR). Microarray results validation by qRT PCR and western blot analysis confirms that A0, but not cisplatin, activates two pathways of the UPR. In particular, IRE1 mRNA is up-regulated, resulting in the increased abundance of the spliced form of XBP1 mRNA that codes for the active transcription factor. The other pathway leads to a rapid phosphorylation of the translation initiator complex subunit eIF2alpha, with the consequent attenuation of protein synthesis and the concomitant preferential translation of the pro-death ER stress responsive proteins ATF4, CHOP and GADD34. Thus, A0, triggering UPR and ER stress in sensitive cancer cells, leads to their death through a paraptotic process and may constitute a new agent to overcome apoptosis resistance.

O.33

ERCC1 and CHK2 in the DNA adduct repair pathway to acquired cisplatin resistance

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Increased DNA-adduct repair is the leading mechanism of acquired cisplatin resistance. Other mechanisms such as decreased cisplatin uptake and sequestration of cisplatin by methallothionein (MT) and glutathione may subsequently become significant. Our previous studies demonstrated that overexpression of ERCC1, the essential component of the nucleotide excision repair (NER), is associated with repair of cisplatin-induced DNA-adduct and with clinical resistance to platinum-chemotherapy. Current investigations provide extensive data on the mechanism of cisplatin resistance via DNA-adduct repair pathway. In response to cisplatin exposure, ovarian cancer A2780/CP70 cells showed increase of ERCC1 mRNA, with increased transcription and increased ERCC1 mRNA half-life. Analysis of the ERCC1 promoter by CAT assay indicates that the region from -220 to -110 appears essential to constitutive expression of ERCC1 gene; a more forward upstream region is responsible for cisplatin-induced ERCC1 overexpression. Identification of functional cis-element in the drug-responsible region of ERCC1 by EMSA revealed that activator AP1 and repressor MZF1 responded to cisplatin stimulation. EMSA with mutated MZF1 probes failed to bind the protein. Overexpression of MZF1 repressed the ERCC1 promoter activity in cisplatin treated cells. These data confirmed that MZF1 is a repressor in regulation of ERCC1 transcription. Upon cisplatin exposure MZF1 mRNA decreased and mRNA levels of c-fos and c-jun increased, suggesting that MZF1 and AP1 coordinately mediate cisplatin-invoked gene expression in these cells. Together, in response to cisplatin treatment, decreased MZF1 and increased AP1 binding activities within the drug-responsible region of the ERCC1 promoter appear to be the leading mechanism of up-regulation of ERCC1 expression. Further, in another investigation of cisplatin-induced molecular signature in A2780 cells, activation of ATM, p53, Chk2, P48, and P21 were observed. Among the activated signals, the Chk2 was identified as an upstream regulator on the ERCC1 recognition/repair pathway. Our data demonstrate that Chk2 is activated and regulated by p53 in wild-type p53 cells. In addition, we found that activated Chk2 can be dephosphorylated by PP2A. In other words, PP2A negatively regulates Chk2 via dephosphorylating phosphorylated Chk2. In summary, our investigations reveal two key factors — ERCC1 and Chk2 — in the DNA repair pathway to cisplatin resistance. These two critical genes can be down-regulated and controlled for potential therapeutic strategies to antagonize cisplatin resistance in treatment of human ovarian cancer.

O.34

Platinum compounds and radiation – understanding the different pathways and translating them to tailored protocols

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Combining concurrent radiation with platinum compounds has been the subject of both preclinical and clinical cancer research for over two decades.[1-5] The property of platinums to enhance the effects of radiation on a variety of tumors has been successfully translated to the treatment of head and neck, non small cell lung (NSCLC), and cervical cancer.

Several molecular pathways accounting for the mechanisms of radiation potentiation and sensitization have been described.[6-9] Platinums create DNA adducts causing cross links which eventually lead to double strand DNA breaks. Such damage to the DNA triggers the cellular mismatch repair (MMR) apparatus, leading to cell cycle arrest and apoptosis. This pathway can be harnessed to enhance radiotherapy by targeting the subset of hypoxic tumor cells, resistant to the traditional effects of radiation.

Noticeably, in tumors with either a genetic defect or a gene rendered nonfunctional due to promoter hyper-methylation in the MMR pathway resistance to both chemotherapies and ionizing radiation occurs. Concurrent administration of both modalities though, has shown to overcome this form of resistance. The mechanism underlying the combined effects includes platinum interference with repair mechanisms of sub lethal radiation damage to DNA and radiation potentiation of the effects of platinum by enhancing uptake and binding to DNA.

Experience from the clinic suggests that many variables govern the success of the combination. Specifically, the kind of platinum compound chosen and its dosing and scheduling during radiotherapy varies among tumor sites.[10-15] The more manageable toxicity profile of Carboplatin makes it a particularly attractive candidate for combined modality treatments.[10] In a phase III CALBG and ECOG study, 283 patients with unresectable stage III NSCLC received radiosensitization with Carboplatin.[11] CR rate was 18% in the chemo-radiation arm versus 10% in the radiation-only arm, without significant difference was detected in the 4 year survival rates (13% versus 10%). Conversely, in cervical cancer the combination of carboplatin and radiotherapy resulted in a survival improvement compared to other chemo-radiation regimes.[13]

Better insight of the genomics and biology of cancer will soon provide clinicians with the necessary rationale to devise multimodality protocols that target individual tumor pathways of resistance. Combinations of platinum agents with ionizing radiation could then be selectively offered to carriers of cancers with genetic and phenotypic characteristics that make them more likely to be vulnerable to this approach.

References

1. Caffo O. *Radiosensitization with chemotherapeutic agents*. Lung Cancer 34, S81-90, 2001.
2. Dewit L. *Combined treatment of radiation and cis-diamminedichloroplatinum(II): A review of experimental and clinical data*. Int J. Radiat Oncol Biol Phys 13, 403-26, 1987.
3. Coughlin CT and Richmond RC Biologic *and clinical developments of cisplatin combined with radiation: Concepts, utility, projection of new trials and the emergence of carboplatin.* Semin Oncol 16, 31-43, 1989.
4. Douple EB. *Interactions between platinum coordination complexes and radiation.* In Interactios Between Antitumor Drugs and Radiation. (B.T> Hill and A. Bellamy, eds.) 171-90. CRC Press, Boca Raton, FL, 1990.
5. Douple EB. *Platinum Chemotherapy for cancer treatment*. In Noble Metals and Biological Systems (R.R. Brooks, ed) 349-76. CRCPress, Boca Raton, FL, 1993.
6. Douple EB, Richmond RC, O'Hara JA et al. *Carboplatin as a potentietor of radiation therapy.* Cancer Treatment Reviews 12 , 111-24, 1985.
7. Yang LX, Douple EB, O'Hara JA et al. *Production of DNA double-strand breaks by interactions between Carboplatin and radiation: a potential mechanism for radiopeotentiation.* Radiation Research 143, 309-15, 1995.
8. Yang LX, Douple EB, Wang HJ. *Irradiation-enhanced binding of Carboplatin to DNA*. Int J Radiat Biol 68, 609-14, 1995.
9. Berry SE and Kinsella TJ. *Targeting DNA mismatch repair for Radiosensitization.* Semin Radiat Oncol 11, 300-15, 2001.
10. Christian MD. *Carboplatin*. Prin Pract Oncol 3, 1-16. 1989.
11. Clmon G, Herndom J, Cooper R, et al. *Radiosensitization with Carboplatin for patients with unresectable stage III non-small-cell lung cancer: A phase III trial of the cancer and Leukemia group B and the eastern Cooperative oncology group.* J Clin Oncol 17, 4-11, 1999.
12. Chougule MD, Akhtar MS, RathoreR, et al. *Concurrent chemoradiotherapy with weekly paclitaxel and carboplatin for locally advanced head and neck cancer: long term follow up of a brown university oncology group phase II study (HN-53).* Head Neck. 2007 Jul 26; [Epub ahead of print]
13. Rose Long-term follow-up of a randomized trial comparing concurrent single agent cisplatin, cisplatin-based combination chemotherapy, or hydroxyurea during pelvic irradiation for locally advanced cervical cancer: a Gynecologic Oncology Group Study. J Clin Oncol 25, 2804-10, 2007.
14. Fournel P, RobinetG, Thomas P. *Randomized phase III trial of sequential chemoradiotherapy compared with concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer: Groupe Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Francais de Pneumo-Cancerologie NPC 95-01 Study.* J Clin Oncol. 23,5910-7,2005.
15. Zatloukal P, Petruzelka L, Zemanova M et al. *Concurrent versus sequential chemoradiotherapy with Cisplatin and Vinorelbine in locally advanced non small cell lung cancer: a randomized study*. Lung Cancer 46, 87-98, 2004.

O.35

Head and neck cancer

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Abstract not received

O.36

Combining platinums: gastrointestinal (non-colorectal) cancer

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Platin-based chemotherapy is the mainstay of care for the perioperative and the palliative treatment of cancer of the esophagus and stomach. Therapy regimens combining two or three drugs are common. The most frequently used platinum analogue is cisplatin. However, multiple phase II studies and two randomized phase III studies have consistently shown an equivalent activity of oxaliplatin. Although oxaliplatin is not yet approved for the treatment of non-colorectal gastrointestinal cancer, more and more oncologists substitute oxaliplatin for cisplatin. The combination partner often fluoropyrimidines. The classical continous infusion schedules of 5-FU are increasingly replaced by using oral fluoropyrimidines like capecitabine or S1. It has been shown that combining a cisplatin-fluorouracil regimen with a third drug, docetaxel, can lead to an increased median survival and a better preservation of quality of life in selected patients who present with metastatic gastric cancer. Perioperative chemotherapy with cisplatin plus fluorouracil with or without epidoxorubicin has also shown to improve the 5-year survival rate in patients with locally advanced gastric cancer and/or adenocarcinoma at the esophagogastric junction (clinical stages II and III, according to UICC criteria). Cisplatin in combination with fluorouracil, but also in combination with irinotecan or a taxane (docetaxel or paclitaxel) is also the backbone of neoadjuvant or defintive chemoradiation therapy in locally advanced squamous cell cancer of the esophagus. First results also indicate a very good activity and tolerability of oxaliplatin in combined modality treatment of the esophagus.
While the role for cisplatin and also oxaliplatin has been well defined by large randomized trials in esophagogastric cancer, the impact on survival in pancreatic cancer remains controversial. Combinations of cisplatin or oxaliplatin with gemicitabine have led to increase response rates compared to gemcitabine alone. However, none of the randomized trials have shown significant improvements in median survival in randomized trials. However, recently published meta-analyses suggest a relevant benefit of giving platin-gemcitabine combinations in those patients with a WHO performance status of 1 or better. Another recently presented randomized study suggests a benefit of second-line treatment with an oxaliplatin-fluorouracil regimen over fluorouracil alone. Interesting phase II data obtained in the adjuvant treatment of resected pancreatic cancer suggest a potential gain in the survival rate of patients receiving a combination of cisplatin, fluorouracil, interferon a and radiation therapy. These data need to be confirmed in a current randomized phase III trial.
Even better measures of supportive care, especially with regards to chemotherapy-induced nausea and vomiting allow for the application of platin-compounds in a majority of the often frail patients presenting with upper gastrointestinal tumors.
Therefore, the role for cis- and oxaliplatin in non-colorectal gastrointestinal expands. Particular emphasis should be placed on further development of highly active and at the same time sufficiently tolerable treatment protocols in the perioperative and curatively intended treatment setting.

O.37

Oxaliplatin-based chemotherapy for colorectal cancer

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For more than 40 years the treatment of colorectal cancer has been based upon the use of 5-Fluoruracil (5-FU) administered according to a variety of schedules, either alone or with several modulators. The response rate has ranged between 10% and 20% with progression free survival (PFS) of 6 months and overall survival in the range of 1 year. The introduction into the clinic of oxaliplatin, a diaminocyclohexane platinum analogue, and the demonstrated synergistic activity when combined with 5FU, led to the popular scheme FOLFOX 4 and its simplified forms, including more recent evolutions with capecitabine as a substitute for leucovorin-modulated 5FU. We learned from several randomised phase III trials that in the advanced setting these combinations can produce a response rate ranging from 37 to 50% with a progression free survival around 8 months. Furthermore, a small percentage of unselected patients initially considered inoperable may become resectable following chemotherapy. In the recently reported EORTC trial (EPOC), a phase III study in which patients with up to 4 liver-only metastases eligible for resection could be randomised to perioperative chemotherapy (6 cycles FOLFOX before liver surgery and 6 cycles following surgery) or to immediate resection, it was demonstrated that patients randomised to the FOLFOX arm had a better PFS at 3 years (35 % vs 28%). Oxaliplatin-based regimens can be further strengthened by the addition of a third component, either a traditional drug such as CPT11 or a targeted agent such as the anti VEGF antibody bevacizumab and the anti-EGFR receptor cetuximab. The sequential administration of all these active agents significantly improved the outcome of advanced colorectal cancer patients with several studies reporting median survivals slightly longer than 2 years. Two large phase III studies (the MOSAIC trial and the NSABP C07) enrolling stage II and III colon cancer patients have consistently demonstrated a 5% absolute improvement in 3-year DFS favouring the oxaliplatin-containing arms. In the MOSAIC trial the improved 3-year DFS translates in a statistically significant better 6-year survival only for stage III patients (73% vs 68.6%). The role of the addition of weekly oxaliplatin to the standard preoperative treatment for locally advanced rectal cancer (continuous infusion 5FU and radiotherapy) is being investigated in an Italian randomised trial.

O.38

New drugs for the management of chemotherapy-induced nausea and vomiting

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Abstract not received

O.39

Importance of cisplatin interaction with organic cation transporters for nephrotoxicity: *in vitro* and *in vivo* studies

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Cisplatin is an effective but highly nephrotoxic antineoplastic agent. In *in vitro* experiments, we clearly identified the human organic cation transporter 2 (hOCT2) as critical transporter for cisplatin nephrotoxicity in isolated human proximal tubules and offer a potential mechanism to reduce nephrotoxicity in clinical practice. This therapeutic approach has been tested *in vivo* using a mouse model of cisplatin nephrotoxicity. Interaction of cisplatin with hOCT2 in kidney or hOCT1 in liver was investigated with the fluorescent cation 4-(4-(dimethyl-amino)styril)-methylpyridinium in stably transfected HEK293 cells and for the first time also in tissues physiologically expressing these transporters, human proximal tubules and human hepatocyte couplets. Cisplatin (100 µM) inhibited transport via hOCT2, but not via hOCT1. In human proximal tubules cisplatin competed with basolateral organic cation transport, while in tubules from a diabetic kidney and in hepatocytes it had no effect. 15 h incubation with cisplatin induced apoptosis in hOCT2 cells, which was completely suppressed by contemporaneous incubation with the hOCT2 substrate cimetidine (100 µM). On the basis of these results, we suggest that a combination of cisplatin with other substrates competing for hOCT2 offers an effective option to decrease nephrotoxicity in the clinical situation. To test this hypothesis, an acute renal failure was induced in FVB mice with 15 mg/kg cisplatin. Four days after cisplatin i.p. injection mice showed significant glucosuria (+844±223%, n=10) accompanied by proteinuria (+58±22%, n=9) and reduction of creatinine clearance (-38±7%, n=7), expressed in % changes compared with the day before injection (day -1). Treatment of mice with cisplatin (15 mg/kg) together with an equimolar dosis of the organic cation cimetidine induced glucosuria (+449±109%, n=8, expressed in % changes compared with day -1), even though at lower level than in cisplatin only-treated mice. Compared with day -1, the creatinine clearance was not changed (-4±10%, n=9), while proteinuria was still induced (+54±20%, n=8).
These findings demonstrate that uptake of cisplatin is mediated by hOCT2 in renal proximal tubules, explaining its organ-specific toxicity. Moreover, *in vivo* combination of cisplatin with the organic cation cimetidine can attenuate some signs of cisplatin nephrotoxicity, but is not able to completely suppress them.

O.40

Treating cisplatin-resistant cancer: a systematic analysis of oxaliplatin or paclitaxel salvage chemotherapy

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OBJECTIVE: To examine the pre-clinical and clinical evidence for the use of oxaliplatin or paclitaxel salvage chemotherapy in patients with cisplatin-resistant cancer.
METHODS: Medline was searched for 1) Cell models of acquired resistance reporting cisplatin, oxaliplatin and paclitaxel sensitivities and 2) Clinical trials of single agent oxaliplatin or paclitaxel salvage therapy for cisplatin/carboplatin-resistant ovarian cancer.
RESULTS: *Oxaliplatin* - Oxaliplatin is widely regarded as being active in cisplatin-resistant cancer. In contrast, the pre-clinical data suggests that there is cross-resistance between cisplatin and oxaliplatin in models which reflect clinical resistance (<10 fold). Oxaliplatin as a single agent had a poor response rate in patients with cisplatin-resistant ovarian cancer (8%, n=91). Oxaliplatin performed better in combination with other agents for the treatment of platinum resistant/refractory cancer suggesting that the benefit of oxaliplatin may lie in its more favourable toxicity and ability to be combined with other drugs rather than an underlying activity in cisplatin resistance. Oxaliplatin therefore should not be considered broadly active in cisplatin-resistant cancer.
Paclitaxel - Pre-clinical data suggests that paclitaxel is active in cisplatin-resistant cancer. 68.1% of cisplatin-resistant cells were sensitive to paclitaxel. Paclitaxel as a single agent had a response rate of 22% in patients with cisplatin-resistant ovarian cancer (n = 1918), a significant increase from the response of oxaliplatin ($p<0.01$). Paclitaxel-resistant cells were also sensitive to cisplatin, suggesting that alternating between agents may be beneficial. Studies of single agent paclitaxel in platinum-resistant ovarian cancer where patients had previously received paclitaxel had an improved response rate of 35.3% n=232 ($p<0.01$), suggesting that pre-treatment with paclitaxel improves the response of salvage paclitaxel therapy. The response rate to paclitaxel/platinum combination regimens in platinum-sensitive ovarian cancer was 79.5% n=88 compared to 49.4% n=85 for paclitaxel combined with other agents ($p<0.001$), suggesting a positive interaction between taxanes and platinum.
CONCLUSIONS: Pre-clinical models reflect the resistance observed in the clinic as the cross resistant agent oxaliplatin has a lower response rate compared to the non-cross resistant agent paclitaxel in cisplatin-resistant ovarian cancer. Alternating therapy with cisplatin and paclitaxel may therefore lead to an improved response rate in ovarian cancer.

O.41

Preoperative radio-chemotherapy of rectal cancer: toxicity and preliminary results with the addition of weekly oxaliplatin

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The standard preoperative treatment of rectal cancer consists of radiotherapy combined with the continuous infusion (7 days a week for six weeks) of fluorouracil (FU) at a dose of 200 mg/m2/die. Platinum compounds can increase the antitumour activity of radiotherapy and they are suitable agents to be combined with FU. Different doses and schedules of FU administration, oral fluoropyrimidines and different doses of Oxaliplatin have been used. We report our experience on the addition of Oxaliplatin to radiotherapy and FU in the preoperative treatment of patients with rectal cancer.
Patients and methods: Patients with locally advanced rectal cancer (cT3-T4 and/or N+) have been treated with preoperative 5-FU (200 mg/m^2/die, continuous infusion) and external beam radiation (45 Gy given to large fields plus a booster dose of 5,4 Gy for a total of 50,4 Gy delivered in 28 daily fraction of 1,8 Gy). Oxaliplatin was given at a dose of 50 mg/m^2 in two hours once weekly. Inclusion and exclusion criteria for patients were standard and written informed consent was obtained before treatment. Surgery was planned 5 weeks after radiotherapy. Toxicity was graded using the NCI-CTC version3. Oxaliplatin was suspended in case of G3 haematological toxicity or G2 neurotoxicity, both Oxaliplatin and FU were suspended for G3 non-haematological toxicity.
Results: From January 2006 to April 2007 13 patients have been treated. All completed radiation treatment, 10 received full dose chemotherapy. Chemotherapy was suspended due to G3 diarrhoea in 3 patients. We observed G2-3 proctitis (which was particularly painful in 3 patients) in 5 patients, G1 allergic reactions in 2 patients and G1 neurotoxicity in 8 patients. 10 patients have been operated: of these 7 had a pathological complete response. In 3 patients histological examination was negative despite the persistence of a palpable mass.
Discussion: Oxaliplatin can be added to standard chemoradiation in the preoperative treatment of rectal cancer. Toxicity is increased and requires careful monitoring in order to avoid any delay or interruption of chemradiation. The present report and literature data indicate that the antitumour efficacy is increased by Oxaliplatin and we look forward to the results of the randomised trial in progress.

O.42

Long-term outcome of unresectable metastatic colorectal cancer (MCRC) patients (pts) treated with first-line Folfoxiri followed by R0 surgical resection of metastases

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BACKGROUND: Prognosis of pts with initially unresectable MCRC can be improved if chemotherapy induces a significant down-sizing of metastatic disease thus allowing an R0 surgical resection of metastases (mts). In particular it has been demonstrated a clear correlation between the activity of the regimen used and the rate of secondary R0 resections (Folprecht et al, Ann Oncol 2005).
METHODS: We studied the triple drug combination Folfoxiri (irinotecan 165 mg/sqm d1, oxaliplatin 85 mg/sqm d1, l-LV 200 mg/sqm d1, 5-FU 3200 mg/sqm 48-h flat continuous infusion starting on d1, repeated every 2 weeks) in phase II and III trials. Overall 196 pts with initially unresectable MCRC and not selected for a neo-adjuvant strategy were treated. This regimen was associated with an elevated activity (response rate ranging from 66% to 72%) and 37 patients (19%) could undergo to a secondary R0 surgery on mts.
RESULTS: Characteristics of the 37 radically resected pts were: median age 64 years (45-73), ECOG PS ≥1 in 11 pts (30%), median CEA 10 ng/ml (1-288), liver involvement ≥25% in 18 pts (49%). Sites of disease were: liver only 25 pts (68%), lung only 4 pts (11%), liver + lymphnodes 5 pts (13%), liver + peritoneum 1 pt (3%), liver + lung 2 pts (5%). Mts were synchronous in 24 pts (65%) and metachronous in 13 pts (35%). There was no perioperative mortality. After a median follow up of 60.5 mos median OS is 41+ mos. The actuarial 5-year survival is 45% from the onset of chemotherapy. In 11 pts progressed after surgery a surgical re-resection and/or radiofrequency ablation was performed.
CONCLUSIONS: These data indicate that Folfoxiri allows an R0 surgical resection in about one out of five pts with initially unresectable MCRC not selected for a neoadjvant approach. Long term survival of resected pts is significant and comparable with the survival of pts resectable up-front. This Folfoxiri regimen should be considered as neo-adjuvant treatment in initially unresectable metastatic colorectal cancer pts. Partially supported by Fondazione ARCO.

O.43

Breast cancer

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For patients resistant to anthracyclines or taxanes no standard regimen exists and platinum-based combinations might have an important role, in particular for.chemotherapy-naïve patients. Cisplatin and carboplatin are active in previously untreated patients with metastatic breast cancer (MBC) with mean response rates (RRs) of 50 and 32%, respectively. In pretreated patients the RR to cisplatin/ carboplatin monotherapy declines markedly to <10%, introducing the concern of chemoresistance. In addition, although platinum compounds have shown activity in MBC since twenty years, the toxicity of these drugs, and of cisplatin in particular, has limited their widespread use in MBC.

Cisplatin and carboplatin have been combined with many other cytotoxic agents. In first-line setting high activity has been observed in combination with taxanes or vinorelbine (RRs consistently approximately 60%),.with infusional 5-FU and epirubicin/paclitaxel/vinorelbine (RRs of around 60 to 80%). In pretreated MBC the combinations of cisplatin taxane/vinorelbine/gemcitabine or carboplatin-docetaxel/vinorelbine yield RRs of 40 to 50%. None of the conventional platinum-based combination regimens in MBC has demonstrated a major impact on patient survival.

The major areas of recent clinical investigation for theese compounds are the first line in MBC, with particular emphasis for specific settings, as HER2 positive and triple negative subgroups, and for their translation into the adjuvant phase.

The use of biological therapies to modulate the RR and hopefully survival has been explored. The synergy demonstrated between platinum compounds, taxanes and herceptin, in preclinical studies suggested the greatest potential for their use in combination. A phase II study by BCIRG employed cisplatin (75 mg/m^2, three cycles) and trastuzumab in pretreated women with MBC and HER-2 overexpressing tumours has reported a 24% RR; this figure is thought to be higher than the RR expected from using the two agents on their own. This synergism, known as receptor-enhanced chemosensitivity, may be explained by data that suggest enhanced sensitivity to cisplatin in the presence of HER-2 downregulation/ inactivation, mediated by reduced repair of cisplatin-induced DNA adducts.

The BCIRG conducted the 101 and 102 phase II studies using the platinum compounds, docetaxel, and trastuzumab combination and concluded that this is a feasible and active combination in patients with advanced breast cancers overexpressing HER2. A subsequent Fase III trial comparing a two arms of combination Taxol-Trastuzumab, with or without CBDCA, confirmed the higher activity of the triple association, with statistical significant advantage for time to progression, compared to the arm without the platinum compound.

The triple drug combination including CBDCA was subsequently tested among the BCIRG 007 study in 232 MBC metastatic breast cancer patients with overexpression/amplification of HER-2 . The results of this study have been

recently reported and no advantage in terms of RR, time to progression and overall survival against a combination without Platinum salts was observed..
The TCH (Docetaxel, Carboplatinum, Trastuzumab) x 6 cycles was tested against AC→T (Adriamycin, Ciclophosphamide, Docetaxel; 4 and 4 cycles) and AC→TH (Adriamycin, Ciclophosphamide, Docetaxel, Trastuzumab; 4 and 4 cycles) in the three arm BCIRG 006 adjuvant Trial.
Two subsequent interim analyses had been presented to the San Antonio Symposium in 2005 and 2006 and the main clinical informations derived at 36 months from this study are the following:

- the addition of Trastuzumab is able to reduce the risk of relapse
- the combination of TCH displayed a similar therapeutic activity to the AC→TH regimen
- significant inferior cardiac toxicity was documented in the arm TCH compared to the former.

A second area of potential investigation for first / subsequent lines of platinum inclusion is the treatment of basal-like and BRCA1 mutated breast cancer patients, showing in the preclinical and initial clinical studies a high sensitivity to this class of agents.
We are waiting the conclusionis of the numerous ongoing randomized trials.
Cautiously, the results in the adjuvant phase need to be confirmed by independent researches and evaluated at longer follow up.
In addition we need further trials to evaluate the consistency of therapeutic effect among the different patient subgroups to confirm the theoretical selectivity on the basis of the biological characteristics and/ or to document the efficacy among different patient subsets.
Clinical data however indicate that the combination with platinum salts is an option today available for women presenting with clinical contraindications or risk factors for anthracycline therapy.

References

Crown,JP. The platinum agents: a role in breast cancer treatment?. Semin. Oncol. 28 (2001), pp. 28–37
Decatris MP, Sundar S, O'Byrne KJ. Platinum-based chemotherapy in metastatic breast cancer: the Leicester (U.K.) experience. Clin Oncol (R Coll Radiol). 2005 Jun;17(4):249-57.
Floros KV, Thomadaki H, Lallas G, Katsaros N, Talieri M, Scorilas A. Cisplatin-induced apoptosis in HL-60 human promyelocytic leukemia cells: differential expression of BCL2 and novel apoptosis-related gene BCL2L12. Ann N Y Acad Sci. 2003 Dec;1010:153-8.
Konecny G, Untch M, Slamon D, Beryt M, Kahlert S, Felber M, Langer E, Lude S, Hepp H, Pegram M. Drug interactions and cytotoxic effects of paclitaxel in combination with carboplatin, epirubicin, gemcitabine or vinorelbine in breast cancer cell lines and tumor samples. Breast Cancer Res Treat. 2001 Jun;67(3):223-33

O.44

Combining platinums with other drugs in ovarian cancer: still the key question after nearly three decades

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Following the studies by Wiltshaw and colleagues in the mid 1970s defining cisplatin's (and subsequently carboplatin's) activity against epithelial ovarian cancer, the platinums were quickly integrated into drug combination regimens. However, it took large trials such as ICON2 and ICON3, and GOG 132, to cast doubt on the contribution of other drugs to the progression-free survival (PFS) achieved by single platinum agents. Although carboplatin + paclitaxel has become the 'standard' initial regimen worldwide (excepting intraperitoneal use of cisplatin in optimally-debulked patients), exposure to paclitaxel in up-front regimens may not be essential. On the other hand, in the recurrence setting, randomized trials have shown superior outcomes when carboplatin is combined with paclitaxel or with gemcitabine, and single arm phase II studies have yielded analogous likely superior results utilizing pegylated liposomal doxorubicin in combination with carboplatin. This seeming paradox is best explained by invoking some degree of platinum-resistance for the other agent to contribute convincingly to outcome.
The dominant role of platinums in ovarian cancer treatment is further reflected by how we clinically classify relapsing ovarian cancer as 'platinum-sensitive' and 'platinum-resistant'. These simple operational definitions do help establish two different paths of decision-making in accordance to the 'platinum-free interval' – by noting if this is greater or less than 6 months. Based on lesser responses to other drug classes as well when the tumor is classified as being platinum resistant, one suspects that biological differences may well exist between those tumors that are classified as resistant and those more readily yield to platinums. In fact, pegylated liposomal doxorubicin by itself appear to yield a superior survival outcome in 'platinum-sensitive' tumors.
Future studies need to explore issues of platinum-resistance at a molecular level. Studies by Dinulescu et al. (1) in an endometrioid ovarian cancer mouse model have recapitulated the platinum sensitivity of these tumors. This laboratory has characterized a subset of cells that may represent stem cells, and contribute to platinum-resistant relapse. Utilizing markers of platinum resistance, may lead to a more rational integration of other active drugs than is currently possible. In addition, new platinums such as oxaliplatin need to be tested for possible activity against this stem cell population.

References

1. Dinulescu D, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T. Role of *K-ras* and *Pten* in the development of mouse models of endometriosis and endometrioid ovarian cancer. Nature Medicine 2005;11:63-70

O.45

The role of platins in endometrial cancer

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20% of women with endometrial cancer will die from it, predominantly from systemic spread. Chemotherapy is therefore needed both for "high risk" women at diagnosis (stages III and IV – all histologies; stage II Clear Cell or grade 3; Papillary Serous or MMMT, irrespective of stage) and for relapsers, unless grade 1 when hormones are a preferable initial option. The most active single agents are the anthracyclines, response rate 17-37%; taxanes 21-67% and platins 13-14%. Combinations have proven to be superior in terms of relapse but not survival:

1994	Thigpen	Doxorubicin vs doxorubicin/cyclophosphamide	RR	22% vs 33%
2003	Aapro	Doxorubicin vs doxorubicin/cisplatin (AP)	RR	17% vs 43%
2004	Thigpen	Doxorubicin vs doxorubicin/cisplatin	RR	25% vs 42%

Taxane/platin containing regimens are the phase III proven best combinations:

2004	Fleming	AP vs AP + paclitaxel + GCSF	RR 34% vs 57%, OS 12 vs 15 m
2003	Weber	AP vs carboplatin-paclitaxel	RR 28% vs 35%

The GOG is currently comparing these 2 "winning combinations" as carboplatin-paclitaxel is a more convenient and less toxic regimen and so would be preferable if equally efficacious. It is likely that other platinum doublets are equally as good. Platin/vinorelbine or carboplatin-liposomal doxorubicin have similar RRs to platin/taxane in phase II studies.

Chemotherapy, predominantly cisplatin-doxorubicin, has improved survival in 3 of the 4 phase III studies conducted (comparing it to irradiation).

			XRT	CHEMO	
2007	Hogberg	Stage I, II, IIIc, IIIc	75%	82%	5 yr PFS
2006	Randall	Stage III/IV	38%	50%	5 yr PFS
2005	Sagae	Stage II/III	64%	84%	5 yr PFS
2006	Maggi	Stage Ic G3; II G3; III	63%	63%	5 yr PFS

Progression still was seen in up to 50% (dependant upon stage). Using "platin/taxane" should improve this somewhat but adding agents directed at molecular targets eg EGFR, VEGF, AKt will be required.

O.46

Lung cancer

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Abstract not received

O.47

Is lipoplatin monotherapy an active alternative in second line treatment of metastatic non small cell lung cancer? A phase II trial

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BACKGROUND: Lipoplatin is a liposome encapsulated form of Cisplatin. Phase I studies on Lipoplatin showed an excellent toxicity profile of the compound. Therefore we performed a phase II trial in pre-treated patients with advanced Non Small Cell Lung Cancer (NSCLC).
METHODS: This was an open label single-arm trial in patients with NSCLC with stage IV disease already pre-treated with first line chemotherpy. 63% of this patients with pre-treated with a regimen platinum containing. Statistical analysis was performed with the SPSS statistical program (version 11.0). Survival curves were estimated by the Kaplan-Meier method. We administered Lipoplatin at the dose of 100 mg/m2 every fourteen days as second line chemotherapy. Primary endpoint was response rate. Secondary endpoint was safety, time to progression and overall survival.
RESULTS: Nineteen patients with stage IV NSCLC median age 64. All of the patients completed at least 6 cycles and were evaluated for response and toxicity. After 19 patients were treated we obtained only one partial response (5,2%) and three stable diseases (15,9%) and the study was stopped. Median time to progression was four months and median survival time was 7 months.
CONCLUSIONS: In this study Lipoplatin as second line drug demonstrated a lower activity in comparison to other drugs used in second line treatment, but the same overall survival. New phase I-II study with escalation of the dosage should be considered.

O.48

Pharmacokinetics of oxaliplatin during open versus laparoscopically assisted Heated Intra-operative intraperitoneal Chemotherapy (HIPEC): an experimental study

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OBJECTIVE. Hyperthermic intraperitoneal chemotherapy (HIPEC) is being evaluated for patients with minimal residual or no residual disease after primary surgery and chemotherapy for stage III ovarian carcinoma. The technical feasability of the laparoscopic approach for HIPEC has been demonstrated in a previous study. An experimental study on the porcine model was carried out in order to compare oxaliplatin pharmacokinetics during a laparoscopic assisted versus the "coliseum" technique for HIPEC.

METHODS. Adult pigs received an HIPEC procedure based on 460 mg/m² of oxaliplatin during 30 minutes with an optimal heated perfusate at 41-43°C. The HIPEC drains were placed in the upper and lower quadrants of the abdomen. Peritoneal fluid and blood samples were collected every ten minutes during the procedure and pharmacokinetics of oxaliplatin was studied.

RESULTS. Two groups of 10 adults pigs were enrolled. All the procedures were successfully completed with an adequate intraabdominal temperature and distribution. No major technical problems were encountered. At the end of the HIPEC, 41.5% of the molecule was absorbed in the laparoscopic group compared to 33.4% in the laparotomy group ($p = 0.0543$). Peritoneal oxaliplatin half-life ($T_{1/2}$) was significantly faster in the laparoscopic procedure (median value of 37.5 min versus 59.3 min, $p = 0.02$). The area under the curve ratio peritoneal / plasma reflects a more important oxaliplatin crossing through the peritoneal barrier in the laparoscopic procedure (ratio: 16.4 in the "closed" procedure versus 28.1 in the open one , $p = 0.03$).

CONCLUSION. This study confirms the technical feasibility and the reliability of the laparoscopic approach for HIPEC, and extends knowledge concerning peritoneal absorption drugs. Oxaliplatin absorption is significantly higher with laparoscopy, regarding time course in the peritoneal perfusion. Clinical application in selected patients may be expected after further experimental investigation designed to define the adequate drug dosage.

O.49

Role of cell cycle arrest in the synergistic interaction between JM118, the active metabolite of satraplatin, and erlotinib

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Satraplatin (JM216) is a novel platinum analog with good oral bioavailability. In preclinical studies, JM118 (the active metabolite of JM216) was active in a number of different tumor models and in cells resistant to cisplatin, carboplatin and oxaliplatin. Erlotinib (Tarceva®) is a potent inhibitor of the tyrosine kinase domain of the epidermal growth factor receptor (EGFR). Since pathways downstream of EGFR may also play a role in the resistance to platinum analogs, we evaluated whether inhibition of this pathway by erlotinib would enhance the sensitivity of non small cell lung cancer (A549, SW1573), colon (Lovo, WiDr) and ovarian (A2780, 2008) cancer cell lines to JM118. The interaction between JM118 and erlotinib was evaluated with the median drug effect analysis. At simultaneous exposure, JM118 and erlotinib were synergistic in A549 and Lovo cells (CI: 0.6, 0.8), and additive in the other cell lines. Pretreatment with JM118 resulted in synergism in all cell lines (CI: 0.5, 0.9). The effect of the single drugs and the combination on cell cycle distribution and expression and phosphorylation of cell cycle proteins (CDC25c, CDK2, CHK1, CHK2 and E2F-1) was investigated. Erlotinib did not show a major effect on the cell cycle, while JM118 caused an S phase block after 24 hours that progressed to a G2/M block after 72 hours. The simultaneous combination induced a similar G2/M accumulation, identical values in the synergistic cell lines and less accumulation in the additive cell lines (77% accumulation with JM118 and 64% with the combination). Preincubation with erlotinib reduced the effect of JM118 in the combination; while erlotinib treatment after JM118 treatment stabilized or even enhanced the JM118 effect. In all cell lines an upregulation of p-CDK2 and p-CHK1 was observed after 24 hours incubation with JM118, accompanied by an increased G1/S accumulation after 24 hours followed by the G2/M arrest after 72 hours. The simultaneous combination also showed an upregulation of p-CDK2 and p-CHK1 expression. In conclusion, JM118 is synergistic with erlotinib in all cell lines, which might be related to JM118 induced G2/M accumulation. Cell cycle effects seem to be the determining factor in the cytotoxic effect of this combination. These preclinical data support ongoing clinical studies investigating the safety and efficacy of the combination of erlotinib and satraplatin.

O.50

The repair capacity for platinum-DNA adducts determines the severity of cisplatin-induced peripheral neuropathy

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The pronounced neurotoxicity of the potent anti-tumor drug cisplatin frequently results in the onset of peripheral polyneuropathy (PNP) which is assumed to be initially triggered by platination products in the nuclear DNA of affected tissues. To further elucidate the molecular mechanisms we have analyzed in a mouse model the formation and processing of the main cisplatin-induced DNA adduct (guanine-guanine intrastrand crosslink) in distinct neuronal cell types by adduct-specific monoclonal antibodies. Comparison of the adduct kinetics in cisplatin-injected mice either proficient or deficient for nucleotide excision repair (NER) functions revealed the essential role of this DNA repair pathway in protecting differentiated cells of the nervous system from excessive formation of such lesions. Hence, chronic exposure to cisplatin resulted in an accelerated accumulation of unrepaired intrastrand crosslinks in neuronal cells of mice with dysfunctional NER. The augmented adduct levels in dorsal root ganglion (DRG) cells of those animals coincided with an earlier onset of PNP-like functional disturbance of their sensory nervous system. Independently from the respective repair phenotype, the amount of persisting DNA crosslinks in DRG neurons at a given cumulative dose was significantly correlated to the degree of sensory impairment as measured by electroneurography. Collectively, these findings suggest a new model for the processing of cisplatin adducts in primary neuronal cells and accentuate the crucial role of effectual DNA repair capacity in the target cells for the individual risk of therapy-induced PNP.

O.51

Allergic reactions

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Abstract not received

O.52

Peripheral neurotoxicity of platinum compounds

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Cisplatin-induced neuropathy is sensory, predominantly characterized by symptoms of large myelinated fibre damage, such as numbness and tingling, paraesthesias of the upper and lower extremities, reduced vibration and position sense perception, reduced deep tendon reflexes, and incoordination with gait disturbance. Occasionally, Lhermitte's sign is reported by patients, suggesting the involvement also of the centripetal branch of the dorsal root ganglia neuron axons in the spinal cord. After completion of cisplatin chemotherapy, only a part of the patients has significant neurotoxic symptoms, whereas 3-4 months later the proportion is definitely higher. This phenomenon (called "coasting") is clinically very relevant, since it makes it difficult to assess the real severity of the dorsal root ganglia neuron damage during cisplatin administration. Resolution or amelioration of symptoms occurs in most of the patients over the next 12 months (despite the fact that abnormal neurological examination is frequently permanent).
Conventional dosages of carboplatin have been associated with a lower risk of peripheral neuropathy than cisplatin. When high dose regimens have been tested in order to achieve a better antineoplastic response, carboplatin peripheral neurotoxicity has become clinically relevant, with symptoms and signs identical to those observed after cisplatin administration.
Besides chronic sensory neurotoxicity, in about 90% of patients, oxaliplatin treatment has been associated with acute neurosensory toxicity including dysaesthesia and paraesthesia. This particular type of neurosensory toxicity predominantly affects the fingers, toes, the pharyngolaryngeal tract, the perioral and oral regions, and it is generally induced or aggravated by exposure to cold. Such symptoms may occur within 30-60 minutes from the beginning or shortly after each course of oxaliplatin. Acute neurotoxicity is generally mild in severity, it disappears within a few hours or days and does not require oxaliplatin treatment withdrawal. In addition to the acute neurotoxic symptoms caused by oxaliplatin, about 10–15% of patients treated with this agent develop moderate neuropathy. The symptoms of chronic neuropathy include non-cold-related dysaesthesia, paraesthesias, superficial and deep sensory loss and, in some cases, sensory ataxia and functional impairment which persists between treatment cycles. Most of these symptoms usually disappear a few months after oxaliplatin withdrawal. Neurophysiological studies in platinum drug-treated patients evidence a reduction in the amplitude of the sensory potentials with minimal changes in the sensory nerve conduction velocity. Pathological examination of sural nerve biopsies has evidenced axonal degeneration without any evidence of primary demyelination.

O.53

Anemia and myelosuppression

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Abstract not received

O.54

Platinum drugs and the children with cancer

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In children Cisplatin is an effective agent in the treatment of testicular tumors and has demostrated activity against neuroblastoma, osteosarcoma, Wilm's tumor, other forms of germ cell tumors, in medulloblastoma and glioma.
The main toxicity of Cisplatin is nefrotoxicity, severe emesis and ototoxicity mainly affecting high frequency. Notably Cisplatin is one of the few agents that has not hematologic toxicity at standard doses.
Cisplatin was initially administered by single dose over 4 to 6 hours with vigorous hydratation and mannitol induced diuresis in order to decrease significantly side effects such as nefrotoxicity and ototoxicity. Fractionation of doses over 5 days may lessen emesis and renal toxicity and decrease ototoxicity without reducing antitumor activity. A number of clinical studies adopt this schedule.
The second generation of platinum-coordination complex Carboplatin was selected out of more than 2000 Cisplatin analouges. Carboplatin is active against neuroblastoma, medulloblastoma, sarcoma and germ cell tumor, however it is sligthly less active than Cisplatin. The pattern of toxicity is however completely different and more favorable with negligible nefrotoxicity, ototoxicity and emesis. On the other hand the dose limiting toxicity of Carboplatin is hematological toxicity.
As compared to adults, in children Carboplatin can be administered at higher doses. Phase II dose was determined to be 400 mg/mq in adults and 560 mg/mq in children.
Since Carboplatin is one of the few drugs where renal clearance correlates with Carboplatin AUC and Carboplatin AUC correlates with hematological toxicity, adoptive dosing formula have been derived to target Carboplatin dose to achieve a target AUC ranging 7 to 10. It's important to note that children formula is different from adult formula.
More recently Oxaliplatin, initially developed to treat adult cancer such as colonrectal cancer, has been utilized in phase I/II studies in children. A phase II trial of Oxaliplatin in combination with Gemcitabine is conducted at the present time in Europe in pediatric patients affected by neuroblastoma, soft tissue sarcoma, brain tumors. Recent results about Gemox protocol "Phase 2 single-arm studies of Gemcitabine in combination with Oxaliplatin in refractory or relapsing pediatric solid tumors" will be shown.
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O.55

Optimizing dose using patient characteristics

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Among the platinum compounds, carboplatin is the only one for which an individual dosing is routinely performed. Indeed, carboplatin clearance (CL) is highly dependent of glomerular filtration rate. Several equations have been proposed to predict carboplatin CL. All are based on patient body weight, age, gender, and serum creatinine (SCr) (i.e., pharmacokinetic covariates). For a decade, this methodology allows the physicians to prescribe carboplatin for patients with poor renal function. However, several factors, particularly SCr, contribute to an heterogeneity between patients and Centers. SCr levels are affected by both the muscle mass and inter-method variability of the assays. Cystatin C (cysC) has been proposed as an alternative endogenous marker of glomerular filtration rate. CysC is a member of the cystatin superfamily of cysteine proteinase inhibitors that is expressed in all nucleated cells. We have shown that CysC is a marker at least as good as SCr to predict carboplatin clearance. Beside the prediction of carboplatin CL, the physicians have to chose a target AUC for dosing of carboplatin. This choice remains largely empirical. We are conducting a multicenter trial to determine patients' characteristics that determine the carboplatin hematotoxicity. The tested pharmacodynamic covariates include demographic, biologic, pharmacogenetic parameters. The population approach used to analyse both neutrophil and platelet counts after carboplatin treatment will be described. The goal of the study is to propose target AUC for the main carboplatin regimen.
By another way, results of drug monitoring of ultrafilterable carboplatin plasma concentrations in patients with dose-intensive therapy will be shown. Motzer et al [J Clin Oncol 2000] proposed an high-dose paclitaxel, ifosfamide, carboplatin (target AUC of 24 mg/mL x minute per cycle), and etoposide (TICE regimen) plus peripheral blood stem-cell rescue for of cisplatin-resistant germ cell tumors. We treat the poor prognosis patients according to the TICE regimen, but using a daily (i.e., Day 1, and Day 2) drug monitoring of carboplatin ultrafiltrable plasma concentrations and individual dosing in order to really obtain the overall AUC. Our experience confirms the favourable results in this group of patients. Beside the expected hematological toxicity, the ototoxicity was the major toxic effect. The dose modifications imposed by the drug monitoring justify this practice.

O.56

Pro! Intraperitoneal (IP) therapy has proven superior to IV therapy for optimally cytoreduced ovarian cancer

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Howell and co-workers first called attention to very favorable results in a subset of patients with recurrent small-volume ovarian cancer receiving IP cisplatin (1). Lack of benefit from such pharmacologic dose-intensification, was attributed to platinum resistance, and up-front aministration of IP therapy was eventually tested in phase III trials (2-4). In these trials, IP platinum-based chemotherapy yielded superior results over the standard IV regimens in three consecutive Gynecologic Oncology Group (GOG) studies (GOG104, GOG114, GOG172), with advantages in progression-free survival (PFS) and overall survival (OS) that hold up in meta-analyses including a number of other smaller trials (5,6).
Resistance to adopting an IP treatment strategy is multifactorial but two objections are raised most often: 1) the GOG trials utilized a flawed control, instead of carboplatin + paclitaxel; and 2) the regimen of GOG172 is too toxic. Selective comparisons across trials overlook evidence favoring IP therapy achieving superior pathologic complete remission rates over IV, the lesser toxicity without IP paclitaxel, and the best PFS with IP cisplatin after IV carboplatin (GOG114). Bevacizumab added to IP or IV regimens is under consideration. However, in the absence of a clinical trial, IP cisplatin must be considered part of the initial treatment strategy for optimally cytoreduced stage III ovarian cancer, in order for patients to derive the maximum benefit achievable to date from either carbo- or cisplatin-based chemotherapy.

References: **1.** Howell SB, Zimm S, Markman M, et al.: Long-term survival of advanced refractory ovarian carcinoma patients with small-volume disease treated with intraperitoneal chemotherapy. J Clin Oncol 5 (10): 1607-12, 1987. **2.** Alberts DS, Liu PY, Hannigan EV, et al.: Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. N Engl J Med 335 (26): 1950-5, 1996. **3.** Markman M, Bundy BN, Alberts DS, et al.: Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 19 (4): 1001-7, 2001. **4.** Armstrong DK, Bundy B, Wenzel L, et al.: Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 354 (1): 34-43, 2006. **5.** Elit L, Oliver TK, Covens A, et al.: Intraperitoneal chemotherapy in the first-line treatment of women with stage III epithelial ovarian cancer: a systematic review with metaanalyses. Cancer 109 (4): 692-702, 2007. **6.** Jaaback K, Johnson N: Intraperitoneal chemotherapy for the initial management of primary epithelial ovarian cancer. Cochrane Database Syst Rev (1): CD005340, 2006.

O.57

Contra! Intraperitoneal chemotherapy should not be adopted as the standard of care in first line chemotherapy for ovarian cancer

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Among strategies recently being explored in clinical trials to improve the outcome of patients with epithelial ovarian cancer, only intraperitoneal (i.p.) chemotherapy has been found to have a significant impact on Progression-free survival (PFS) and Overall survival (S). Although three phase III trials have documented a statistically significant advantage with this approach, the oncology community has not universally accepted i.p. chemotherapy as new standard first line treatment for advanced ovarian cancer. Specific criticisms have been moved to each of these trials and contribute to divergent interpretation of clinical data with subsequent scepticism and lack of acceptance by many clinicians. The main criticism to GOG 104 was the use of an obsolete standard arm(cisplatin-cyclophosphamide): therefore the observed benefit of i.p. therapy could have been achieved more easily with the intravenous (i.v.) administration of cisplatin and paclitaxel. GOG 114 was criticised because the study design included additional variables such us two cycles of carboplatin AUC 9 prior to i.p. therapy, therefore the contribution of i.p. therapy could not be clearly defined. Moreover only PFS was improved and the toxicity was unacceptable. Additional variables were also introduced in GOG 172 (platinum dose, paclitaxel dose and schedule) and this fact confounds the ability to attribute benefit solely to i.p. delivery. Moreover, several difficulties were observed in administering total of 6 cycles to substantial percentage of patients , due to toxicity and impaired quality of life. Open questions include outcome in patients who did not complete i.p. regimen and reasons for the much greater advantage in S compared to PFS for the i.p. group, with median S in the control group far below expectation. Moreover salvaged therapy was uncontrolled and unreported. In conclusion, no optimal i.p. chemotherapy has been established to date, since experimental arms were different among the three trials , too toxic in GOG 104 and intolerable in > 50% of patients in GOG 172. To convince the oncology community about the value of i.p. chemotherapy a feasible i.p. regimen should show a clear benefit over the current best standard of care in a properly designed randomized clinical trial . This evidence is not available as yet.

O.58

IP therapy - Where do we go from here?

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Intraperitoneal therapy has now been demonstrated in multiple randomized trials to improve the outcome of chemotherapy for patients with optimally debulked or small volume ovarian carcinoma. However, the magnitude of the benefit of IP therapy is limited and it is associated with new types of adverse events.
The core pharmacologic concept upon which IP therapy is founded is that this route of administration will deliver more cytotoxic drug to the tumor. Poor drug penetration from the surface of tumor nodules into their interior is a major factor that limits such a benefit. In combination with adherence to the basic pharmacologic principles that govern the advantage of IP therapy, substantial improvements in the therapeutic index are potentially possible due to new information about the cellular pharmacology of the platinum-containing drugs and novel targeted delivery systems capable of directing toxins to the tumor. One way of enhancing penetration is to maintain very high concentrations of drug in the peritoneal cavity for long periods of time. This is not possible with cisplatin because the AUC ratio is only in range of 15. However, when using drugs whose systemic clearance far exceeds their peritoneal clearance it is possible to maintain IP concentrations that are >1,000-fold higher than plasma levels. Under these circumstances, effective concentrations can be maintained in the peritoneal cavity for many days with little systemic toxicity. A second strategy for increasing tumor nodule penetration is to modify the rate at which drug diffusing in from the surface is washed out of the tumor via capillary flow. Bevacizumab has activity as a single agent in ovarian cancer suggesting that it reduces capillary flow in tumor nodules and thus may significantly enhance tumor penetration. This is a high priority for investigation. Cisplatin down-regulates the expression of the influx transporter CTR1 that is responsible for much of the initial rate of platinum accumulation in tumor cells. This can be prevented by pharmacologically disabling the proteosome. Pre-treatment of human ovarian cancer cells with bortezomib prevents cisplatin-induced down-regulation of CTR1 and increases cisplatin uptake. In murine models of ovarian cancer, bortezomib has favorable IP kinetics producing an AUC ratio of ▫67. Thus, the combination of cisplatin and bortezomib is an attractive strategy for increasing tumor penetration. A series of peptides have recently been identified that cause small particles to adhere to ovarian cancer cells very tightly. Studies are underway to utilize cisplatin-loaded nanoporous silicon particles coated with these peptides to create extremely high local concentrations of cisplatin at the tumor nodule surface so as to drive more drug into the nodule down a steep concentration gradient while at the same time limiting the amount of cisplatin reaching the systemic circulation per unit time. In conclusion, there is a reasonable basis for the expectation that the unique biology of ovarian cancer growth on peritoneal surfaces, coupled with the special pharmacokinetic features of IP drug delivery, can be exploited to further improve the overall efficacy and safety of IP therapy.

O.59

Preclinical status and clinical development of new non platinum antitumor drugs

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While in the platinum field interest in active non-classic platinum complexes is increasing because of their inability of resembling cisplatin in their cellular effects and mode of action, some metals other than platinum inherently have proper preconditions for this purpose. Differences in coordination chemistry form the basis for a diversity of pharmacologically relevant interactions with biomolecules, raising the hope that compounds of these metals may be prove clinically active in malignancies that cannot be treated effectively with platinum complexes.
Complexes of ruthenium constitute a divergent class of agents not apprehensible by simple analogies with platinum drugs. Ruthenium is redox active under physiological conditions and capable of interfering with iron-related processes, predestining it for exploiting the pathophysiological conditions of solid tumors. These properties probably contribute to the tumor-selective activity of indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019, FFC14A), which can be delivered to tumor cells by transferrin-mediated uptake and can be activated by reduction under hypoxic conditions. A phase I dose escalation study in patients with advanced solid tumors showed the high tolerability of this compound and disease stabilizations in the majority of patients.
It has long been recognized that gallium exerts antineoplastic effects in the form of simple salts such as gallium nitrate, and the interference with cellular iron-dependent metabolism seems to be crucial for this activity. Orally applicable complexes such as tris(8-quinolinolato)gallium(III) (KP46, FFC11) offer the possibility of a more convenient, less toxic and more tumor-selective use of gallium. A clinical phase I study confirmed the high tolerability of KP46, and with one partial response and two cases of disease stabilization this is the first metal compound to show at least preliminary evidence of clinical activity in renal cell carcinoma.

O.60

Clinical and pre-clinical status of emerging platinums

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This contribution will summarize recent work on developments of new platinum anticancer agents. A broad survey of work will highlight new clinical developments on satraplatin and picoplatin. In pre-clinical surveys published by us and others, advances in understanding of uptake mechanisms and interactions with signal transduction inhibitors will highlight the need for further research to identify new platinum agents and new
platinum agent combinations with clinical anticancer potential.

O.61

Non-coordinating metal complexes that bind to nucleic acids

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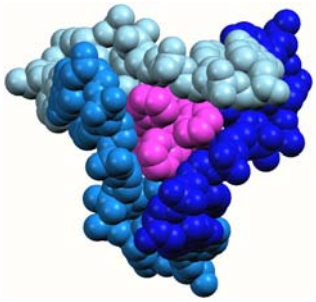
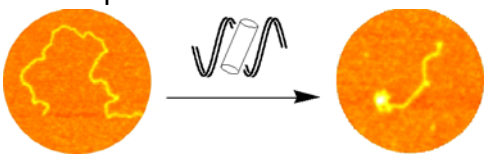


Recognition of a DNA 3-way junction

Within biological systems, sequence specific DNA recognition is achieved by the surface motifs of proteins, which generally interact non-covalently with the major groove of DNA. Such protein recognition of DNA contrasts with that of synthetic small molecule drugs. Being smaller in scale than proteins, they tend to act via intercalation, target the minor groove or bind to the bases as seen for platinum metallo-drugs. Our team has been developing metal-based agents which recognise DNA non-covalently. A particular success has come from metallo-supramolecular cylinders[1] which are a similar size and shape to the zinc finger motifs found in certain DNA-recognition proteins. We have demonstrated that these synthetic tetracationic supramolecular cylinders bind strongly (binding constant > 10^7 M^{-1}) and non-covalently to DNA in the major groove and induce dramatic and unexpected intra-molecular DNA coiling that is unprecedented with synthetic agents and somewhat reminiscent of the effect of histones. Still more excitingly they can bind DNA Y-shaped junctions.[2] This new and unprecedented mode of DNA recognition could transform the way that scientists think about how molecules can bind DNA, and opens the possibility of designing agents that recognise DNA replication forks thereby specifically targetting rapidly dividing cells. The effects of these new agents on cancer cells will be described.[3]



AFM images showing the dramatic intra-molecular coiling induced by the cylinder

References

1. M.J. Hannon, *Chem. Soc. Rev.*, 2007, 36, 280-295; M.J. Hannon, V. Moreno, M.J. Prieto, E. Molderheim, E. Sletten, I. Meistermann, C.J. Isaac, K.J. Sanders, A. Rodger, *Angew. Chem., Intl. Ed. Engl.*, 2001, 40, 879; I. Meistermann, V. Moreno, M.J. Prieto, E. Moldrheim, E. Sletten, S. Khalid, P.M. Rodger, J.C. Peberdy, C.J. Isaac, A. Rodger, M.J. Hannon, *Proc. Natl. Acad. Sci., USA.*, 2002, 99, 5069; L.J. Childs, J. Malina, B.E. Rolfsnes, M.J. Prieto, M.J. Broome, M. Rodger, E. Sletten, V. Moreno, A Rodger, M.J. Hannon, *Chem. Eur. J.* , 2006, 12, 4919-4927.
2. A. Oleksi, A.G. Blanco, R. Boer, J. Uson, J. Aymami, A. Rodger, M.J. Hannon, M. Coll, *Angew. Chem. Int. Ed.* 2006, 45, 1227; J. Malina, M. J. Hannon, V. Brabec, *Chem. - Eur. J.*, 2007, 14, 3871-3877; L. Cerasino, M. J. Hannon, E. Sletten, *Inorg. Chem.*, 2007, 46, 6245-6251.
3. G. I. Pascu, A. C. G. Hotze, C. Sanchez Cano, B. M. Kariuki, M. J. Hannon, *Angew. Chem., Intl. Ed.*, 2007, 46, 4374-4378; A.C.G. Hotze, B.M. Kariuki, M.J. Hannon, *Angew. Chem., Intl. Ed.*, 2006, 45, 4839-4842

O.62

Platination of telomeric DNA: towards the destabilisation of telomers

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Telomeres are DNA-protein structures that cap chromosome ends protecting them from degradation and end-to-end fusions. Telomeric DNA consists of highly repetitive short sequences of guanines residues $(TTAGGG)_n$ followed by an overhang at the 3' end of the G-rich strand. In the presence of monovalent cations, Na^+ or K^+, it is able to adopt stable G-quadruplex structures that were shown to inhibit telomerase, an enzyme involved in the immortalisation of cancerous cells. In mammals, two telomeric DNA-binding proteins termed TRF1 and TRF2 have been identified to bind to double-stranded TTAGGG repeats. TRF1 functions as a negatively regulator of telomere length and TRF2 protects chromosome ends. As platinum complexes bind preferentially to guanines, telomeric DNA is a potential target. First, we have shown that cisplatin and some derivatives were able to cross-link the quadruplex structures of the single-stranded telomeric DNA $AG_3(T_2AG_3)_3$ in the presence of Na^+ or K^+ (1, 2, 3, 4). Then, we have shown that the platination of the double-stranded telomeric DNA on either one of the four triplet of guanines of $(T_2AG_3)_4$ impeded the binding of TRF1 and TRF2 (5). Brought together, these data suggest that the platination of telomeric DNA could interfere with the telomere integrity either by inhibiting telomerase or by impeding the binding of TRF1 and TRF2.

Reference

1. Redon, S., Bombard, S., Elizondo-Riojas, M. A. and Chottard, J. C. (2003).Nucleic Acids Res., 31, 1605-1613
2. Ourliac-Garnier I., Elizondo-Riojas M. A., Redon S., Farrell N.P. and Bombard S., (2005) *Biochemistry*, **44**, 10620-10634
3. Ourliac-Garnier, I and Bombard, S (2007) GG sequence of DNA and the human telomeric sequence react with cis-diammine-diaquaplatinum at comparable rates. *J. Inorg. Biochem*. **101**, 514-524
4. Bertrand, H, Bombard, S, Monchaud, D, and Teulade-Fichou, M.P (2007). Platinum-Quinacridine Hybrid as G-Quadruplex-Ligand. *J. Biol. Inorg. Chem*. **12**, 1003-1014
5. Ourliac-Garnier, I, Giraud-Panis, M.J., Bombard S (2007). Platination of telomeric DNA by cisplatin disrupts recognition by TRF2 and TRF1, *in preparation*.

O.63

Nucleobase quartets as novel targets for metal drugs

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Tetrastranded nucleic acid elements, which cap the ends of the chromosomes (telomers) or occur in regulatory regions of the genome, appear to have important physiological functions, and have become major targets for antitumor strategies in recent years.[1] Guanine quartets represent the most stable entities in these structures, frequently supporting the existence of other base quartets or triplets. Metal ions are integral parts of all these structures. The lecture will provide a brief overview of the field, point out strategies to generate artificial analogues, and highlight the potential of metal complexes to interact with quadruplex nucleic acids.

1. See various articles in "Quadruplex Nucleic Acids", S. Neidle and S. Balasubramian (eds), RSC Publishing, RSC Cambridge, 2006.

O.64

Interaction of the DNA mismatch repair protein MutS with cisplatin-modified DNA: molecular basis for DNA damage signalling

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The DNA mismatch repair (MMR) pathway ensures the fidelity of DNA replication and initiates the cellular response by signaling certain classes of DNA damage produced by chemotherapeutic agents such as cisplatin. It is thought to mediate cytotoxicity through recognition of cisplatin lesions followed by yet undefined MMR-dependent biochemical activities which could initiate a signal for further activation of downstream events. We will present interaction studies of cisplatin-modified DNA with the bacterial MMR protein MutS, which is implicated in DNA mismatch recognition and in ATP-dependent steps of the repair initiation.
Site-specifically modified DNA oligonucleotides containing each of the four cisplatin cross-links or various compound lesions (a lesion in one strand and a mismatched base opposite to it in the other) were used to define MutS recognition properties with cisplatin damage DNA. The strength of interaction of MutS with a set of cisplatin compound lesions suggests that such lesions are more plausible critical lesions for MMR-mediated cisplatin cytotoxicity (1).
Another point that we 'll address here is to know whether a compound lesion, i.e. a mispaired thymine opposite the 3' platinated guanine of the major cisplatin intrastrand cross-link, could modulate or not important steps of MutS-dependent DNA mismatch repair initiation such as ATP-dependent DNA release, DNA-dependent increase of ATP hydrolysis and protein conformational changes upon DNA binding in the presence of ATP. We 'll show for the first time that several ATP-dependent properties of MutS are specifically associated with a cisplatin compound lesion and are distinguishable from those exhibited with a mispaired base (2). Importantly, a cisplatin compound lesion can stabilize a MutS intermediate species in the presence of ATP. The formation of such an unusual stable nucleoprotein intermediate could be viewed as a new MMR function devoted to cisplatin-DNA damage signaling. Our data will be discussed in the context of the existing models linking MMR function with cisplatin damage signaling.

References

1) L. Fourrier, P. Brooks and J.-M. Malinge, J. Biol. Chem. 278, 21267 (2003).
2) Y. Sedletska, L. Fourrier and J.-M. Malinge, J. Mol. Biol. 369, 27 (2007).

O.65

Nucleotide excision repair of DNA cross-links of platinum complexes

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The major intrastrand cross-links of antitumor cisplatin and its analogues distort DNA conformation, are recognized by a number of damaged-DNA binding-proteins and can be removed from DNA by several repair pathways. Nucleotide excision repair (NER) is a major cellular defense mechanism against the toxic effects of cisplatin and other platinum-based chemotherapeutic agents. It has been found using various NER systems that intrastrand cross-links of cisplatin are efficiently repaired (1). Importantly, the repair of major intrastrand cross-links of cisplatin is blocked upon addition of an HMG domain protein (HMG = high mobility group). According to one hypothesis of the mechanism underlying antitumor effects of cisplatin (repair shielding model) (1, 2), which might be effective in a certain type of tumor cells, the proteins binding to DNA damaged by cisplatin block DNA adducts of cisplatin from damage recognition needed for repair. In this way these adducts can persist for sufficient time, which potentiates the antitumor effect of the drug.
The examinations of NER of DNA adducts of several new antitumor platinum complexes and model compounds have revealed that some of these adducts are not removed so readily by NER as intrastrand adducts of antitumor cisplatin (3, 4). Similarly, some new antitumor platinum compounds form bulky ternary DNA-Pt-protein complexes which are not removed from DNA by NER systems (5, 6). Hence, to exert antitumor effect, these platinum adducts would not have to be shielded by damaged-DNA recognition-proteins so efficiently as plain intrastrand cross-links of cisplatin. It is reasonable to suggest that these adducts can persist even without being shielded by DNA-binding proteins, which may potentiate their toxicity toward tumor cells sensitive to these new platinum drugs.

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References
1. Zamble, D.B., Mu, D., Reardon, J.T., Sancar, A., Lippard, S.J. (1996) Biochemistry, 35, 10004-10013.
2. Wei, M., Burenkova, O., Lippard, S.J. (2003) J Biol Chem, 278, 1769-1773.
3. Kasparkova, J., Novakova, O., Marini, V., Najajreh, Y., Gibson, D., Perez, J.-M. and Brabec, V. (2003) J Biol Chem, 278, 47516-47525.
4. Marini, V., Christofis, P., Novakova, O., Kasparkova, J., Farrell, N., Brabec, V. (2005) Nucleic Acids Res, 33, 5819-5828.
5. Novakova, O., Kasparkova, J., Malina, J., Natile, G., Brabec, V. (2003) Nucleic Acids Res, 31, 6450-6460.
6. Chvalova, K., Brabec, V., Kasparkova, J. (2007) Nucleic Acids Res 35, 1812-1821.

O.66

Interactions of Pt complexes with cell extracts

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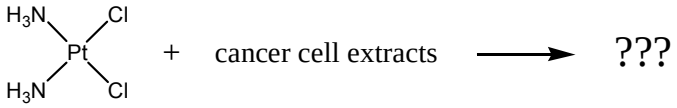
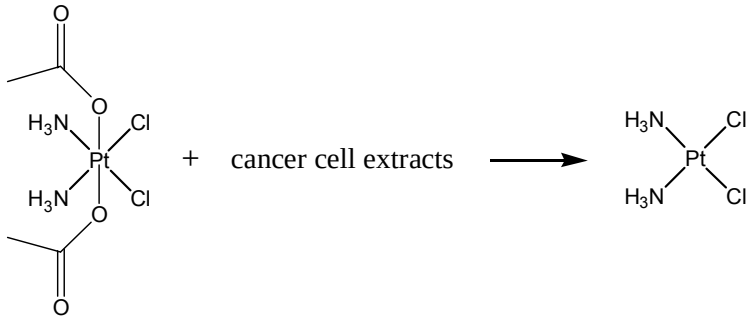
The anticancer drug cisplatin is one of the three most extensively used anticancer drugs. Cisplatin is administered intravenously, but only a minority of the molecules find their way to the cancer cells, penetrate them and react with one or more cellular components. Much of the understanding about the mechanism of action of the drugs is derived from data gathered from aqueous model systems simulating "physiological conditions". We have chosen to use extracts of cancer cells as models for the cytosols of cancer cells. We prepared cisplatin labeled with ^{15}N and *cis*-[$PtCl_2(^{13}CH_3NH_2)_2$] labeled with ^{13}C for 2D-HSQC NMR studies. We compared the interactions between cisplatin and extracts of cancer cells sensitive and resistant to cisplatin. Interestingly we did not observe any aquated species of cisplatin nor interactions with sulfur nucleophiles.



Pt(IV) complexes are inert octahedral compounds that are often used as prodrugs for the more cytotoxic Pt(II) analogues. Upon reduction, the octahedral Pt(IV) loses both it axial ligands generating the tetracoordinate square planar Pt(II) complex. We began studying the stability of Pt(IV) complexes with axial acetate ligands in extracts of different types of cancer cells. Towards that end we prepared a Pt(IV) complex *ctc*-[$PtCl_2(OAc)_2(NH_3)_2$] labeled with ^{13}C so that the reduction can be monitored by 2D-HSQC. We have seen that the rate of reduction of the Pt(IV) complex in the extract depends on the type of the parent cancer cell line.



O.67

Platinum drug uptake in tumours, tumour models and cells: imaging and modification

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The selective activation of prodrugs in tumour environments is one of the most promising approaches for reducing the toxicity of anticancer drugs. However, the rational design of such prodrugs requires a knowledge of their uptake and the location of the site of activation in the tumour and this has been lacking to date. We have been employing a combination of X-ray fluorescence mapping (SRIXE), XANES spectroscopy, and confocal fluorescence microscopy to monitor the uptake, metabolism, and activation of platinum(IV) and cobalt(III) prodrugs.
SRIXE mapping and XANES has allowed us to determine the location and oxidation state of platinum complexes in a variety of tumour models and has revealed significant problems with uptake into tumour spheroid models. These methods require access to synchrotron radiation and therefore we have been conducting parallel experiments using confocal fluorescence microscopy. Fluorescence tagging has proven to be an effective means for monitoring the cellular uptake, localisation, and site of action of these agents. Fluorescence microscopy has been employed to study the cellular distribution of several platinum-anthraquinone complexes by ourselves and others and has revealed that sub-cellular organelle localisation of such compounds is probably responsible for lower than expected activity. We have also shown that platinum and cobalt are able to alter the fluorescence of bound fluorophores and this has allowed us to monitor the coordination and oxidation state of both metals in cellular environments. Using changes in fluorescence, we have been able to visualise differences in uptake and distribution of the metal complexes and the fluorescent ligands.

O.68

Activation and cytotoxicity of light-activated diazido-Pt(IV) complexes as potential anticancer drugs

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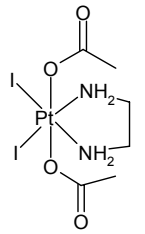
The development of photoactivatable prodrugs of platinum-based antitumor agents is aimed at increasing the selectivity and hence lowering toxicity of this important class of antitumor drugs.[1] These drugs could find use in treating localized tumors accessible to laser-based fiber-optic devices. Pt^{IV} complexes appeared attractive because these octahedral complexes are usually substitution inert and require reduction to the Pt^{II} species to become cytotoxic. Based on the knowledge of Pt^{IV} photochemistry, Pt^{IV} analogs of cisplatin, $[Pt(en)Cl_2]$ and transplatin were designed, synthesized and investigated for their ability to be photoreduced to cytotoxic Pt^{II} species. Two classes of photoactivatable Pt complexes have been looked at thus far: diiodo-Pt^{IV} and diazido-Pt^{IV} diam(m)ine complexes. The first generation, diiodo-Pt^{IV} complexes, represented by *cis, trans*-$[Pt(en)(I)_2(OAc)_2]$ (**1**), react to visible light by binding irreversibly to calf thymus DNA and forming adducts with 5'-GMP in the same manner as $[Pt(en)Cl_2]$. Furthermore, the photolysis products are cytotoxic to human cancer cells *in vitro*. However, these complexes were too reactive towards biological thiols (i.e., glutathione), which rapidly reduced them to cytotoxic Pt^{II} species, thus making them unsuitable as drugs. The second generation, diazido-Pt(IV) complexes, represented by *cis, trans, cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ (**2**) is also photosensitive, binding irreversibly to calf thymus DNA and forming similar products with DNA and 5'-GMP in the presence of light as the respective Pt^{II} complexes. Moreover, they are stable to glutathione and thus show very low dark cytotoxicity. Light of λ_{irr} = 366 nm activates these complexes to cytotoxic species that effectively kill cancer cells by destroying their nuclei, leaving behind shrunken cell ghosts. Interestingly, the all-trans complex, *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(NH_3)(C_5H_5N)]$ (**3**), which can also be photoactivated to Pt species that irreversibly platinate calf thymus DNA, is the most potent of all the light-activated Pt^{IV} complexes investigated to date. Photoactivatable Pt antitumor agents thus represent a promising area for new anticancer drug development.



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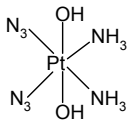

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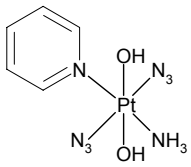

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References

1) Bednarski, PJ, Mackay, FS, Sadler, PJ Anti-Cancer Agents in Medicinal Chemistry, 2007, 7: 75-93.

O.69

Platinum(II) metallointercalators with potent in vitro cytotoxic activity

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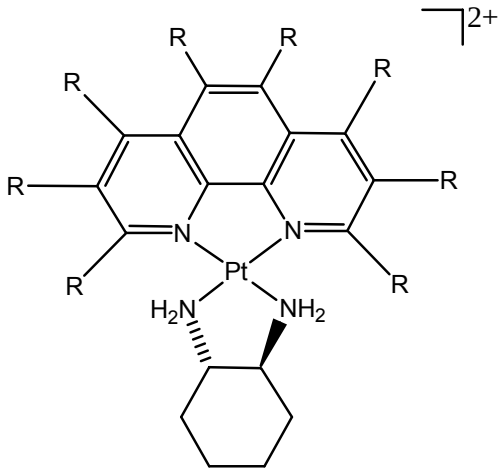
Platinum(II)-based metallointercalators have been synthesised that utilise substituted 1,10-phenanthroline (phen) ligands, achiral (ethylenediamine, en) and the chiral ancillary ligands (1*S*,2*S*-diaminocyclohexane (*S,S*-dach) and 1*R*,2*R*-diaminocyclohexane (*R,R*-dach)). Their binding studies and cytotoxicity in the L1210 murine leukaemia and human cell line using growth inhibition assays were determined. The most cytotoxic metal complexes are [(5,6-dimethyl-1,10-phenanthroline)(1*S*,2*S*-diaminocyclohexane)platinum(II)]$^{2+}$ (56MESS), [(5-methyl-1,10-phenanthroline)(1*S*,2*S*-diaminocyclohexane)platinum(II)]$^{2+}$ (5MESS) and [(1,10-phenanthroline)(1*S*,2*S*-diaminocyclohexane)platinum(II)]$^{2+}$ (PHENSS). All DNA intercalators tested to date which contain *S,S*-dach ancillary ligands display higher cytotoxicity than cisplatin in the L1210 murine leukaemia cell line. Our lead drug 56MESS is about 100-fold more cytotoxic than cisplatin.



POSTER PRESENTATIONS

P.01a

Platinum release characteristics of Prolindac (AP5346)

Sood P, Weiss RC, Zarzycki R, Nowotnik DP
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ProLindac (AP5346) consists of DACH-platinum attached through a triglycyl-amidomalonate linker to a water soluble, biocompatible 2-hydroxypropylmethacrylamide (HPMA) polymer backbone[1]. ProLindac was designed as a prodrug, wherein the polymeric delivery system utilizes the EPR effect (enhanced permeability and retention; whereby macromolecules take advantage of the leaky endothelial cell layer of tumor blood vessels to penetrate tumors if preference to normal tissues), while the DACH platinum attached to the polymer has no activity until it is detached from the polymer. In eleven mouse tumor models, ProLindac has demonstrated at least equivalent efficacy to that of equitoxic doses of oxaliplatin, and in the majority of cases, ProLindac displayed superior or significantly superior efficacy. Promising preliminary evidence of efficacy was obtained in a Phase 1 clinical study of ProLindac[3], and ProLindac is currently being examined in a Phase 2 study in ovarian cancer patients[4].
The release of platinum from the polymer was designed to occur in acidic environments such as the extracellular space of hypoxic tumors and/or the intracellular lysosomal compartment[2]. We have shown previously an inverse relationship between pH and platinum release rate in the pH range 5.4 to 7.4[1]. Minimal platinum release occurs at either of these pH levels in the absence of ligating nucleophiles, such as chloride. The release of DACH-platinum from the polymer has now been studied in various environments. Sulfur-containing molecules of biological significance such as cysteine, cystine, glutathione, and methionine can also catalyze the displacement of DACH platinum from the polymer. Results of displacement studies using these ligands individually and in combination will be presented.

References

1. P. Sood, K Thurmond, J. Jacob et al., Bioconj. Chem. 2006, 17:1270-1279.
2. J. Rice, J. Gerberich, D. Nowotnik, S. Howell, Clin Cancer Res. 2006, 12:2248-2254.
3. M. Campone, J.M. Rademaker-Lakhai, J. Bennouna. et al., Cancer Chemother Pharmacol, 2007, 60:523-533.
4. http://www.accesspharma.com/pr/070820.pdf

P.02a

Cell death, apoptosis and inhibition of the thioredoxin reductase activity induced in prostate cancer cells by gold(III)dithiocarbamate complexes

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The treatment of a variety of cancers by platinum derivatives has encouraged the continuous investigation of alternative metal-based drugs with good antiproliferative activity and lack of systemic toxicity. The development of new gold(III) complexes, characterized by an adequate stability under physiological conditions, higher cytotoxicity and fewer side effects than existing metal anticancer drugs, and able to overcome the resistance to cisplatin, has been actively obtained in recent years.[1,2,3]

In the present study, we have investigated the antineoplastic activity and the molecular mechanism of action of two new gold complexes, on the androgen independent prostate cancer cell line PC3, representing the prototype of advanced, clinically untreatable stages of prostate progression. These two very promising gold(III) dithiocarbamate derivatives $[(DMDT)AuCl_2]$ (dichloro(N,N-dimethyldithiocarbamato-kS,kS')gold(III)) and $[(ESDT)AuBr_2]$ (dibromo[ethyl-N-(dithiocarboxy-kS,kS')]-N-methylglycinato gold(III)) contain S-donor ligands able to strongly bind the metal center preventing interactions with sulfur containing enzymes, which are believed to be at the basis of the toxicity of platinum(II) based drugs. Both compounds suppressed, in a dose dependent manner, proliferation of PC3 cells, with ID_{50} values of about 1 μM and lower than that of the reference drug Cisplatin (4 μM). Exposure to cytotoxic concentration of gold(III) dithiocarbamate derivatives determined: induction of apoptosis (as determined by a higher induction of APO2.7, phosphatidylserine exposure, mitochondrial membrane permeabilization, release of Cytocrome c and DNA fragmentation), down-regulation of the anti-apoptotic molecule Bcl-2 and up-regulation of the proapoptotic molecule BAX. Furthermore our gold(III) compounds inhibit the activity of thioredoxin reductase enzyme. This finding is highly significant as the increasing expression of thioredoxin/thioredoxin reductase system is associated with aggressive tumour growth and decreased patient survival.

Finally gold(III) compounds remarkably inhibited the migration of tumor cells, as measured in a scratch wound assay.

Altogether, our results indicate that these new gold(III) dithiocarbamate derivatives are of undoubted therapeutic relevance and might represent new potentially active agents for the management of prostate cancer.

References

1. Ronconi, L., Giovagnini, L., Marzano, C., Bettio, F., Graziani, R., Pilloni, G., and Fregona, D.(2005). Inorg. Chem. 44, 1867-1881.
2. Ronconi, L., Marzano, C., Zanello, P., Corsini, M., Miolo, G., Maccà, C., Trevisan, A., and Fregona, D. (2006). J. Med. Chem. 49, 1648-1657.
3. Aldinucci, D., Lorenzon, D., Stefani, L., Giovagnini, L., Colombatti, A., and Fregona, D.(2007). Anti-Cancer Drugs 8, 323-332.

P.03a

Novel platinum complexes of potential application in cancer chemotherapy

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M.D. Anderson Cancer Center, Houston, Texas US

Novel platinum (IV) complexes of the type [A Pt (V1)(X2) Y2] were synthesized, where A=cis-1,4-diaminocyclohexane, X= acetato, propionato, or butyrato and Y = Cl. The IC50 of complexes L121/0 cell line was in the range of 0.003 – 0.15 μM (cisplatin, 0.18 μM) against the L1210/0 cell line, 0.13 – 8.0 μM (cisplatin 35 μM) against the 200 fold cisplatin-resistant L1210/DDP, and 0.02 – 3.1 μM (cisplatin 1.8 μM) against the 40 – fold tetraplatin – resistant L1210/DACH. Resistance factors for complexes were 50 -60 in L1210/DDP line and 7 – 21 in L1210/DACH line. In vivo activity (% T/C) of the complexes against L1210/0 was in the range of 140-500 (cisplatin: % T/C = 195) at MTD (5-6.25 mg/kg ip, d.1,5,9). Intracellular accumulations (100μM, 2hr, 37°C) were 1.5 – 3.0 fold greater for the 1,4-DACH-acetato-Pt (IV) congener vs cisplatin in L1210/0 and L1210/DDP cells, but levels were similar for the two agents in L1210/DACH cells. Interestingly, total DNA adducts were 1.5 – 3.0 fold lower for this acetato complex vs cisplatin in all three lines indicating that fewer adducts are required with the new analog to induce cell kill. Thus, 1,4 DACH-Pt(IV) agents with axial carboxylato ligands display excellent potencies and partial cross--resistance properties.

P.04a

New platinum(II) metallointercalators with potent cytotoxic activity on human cancer cells

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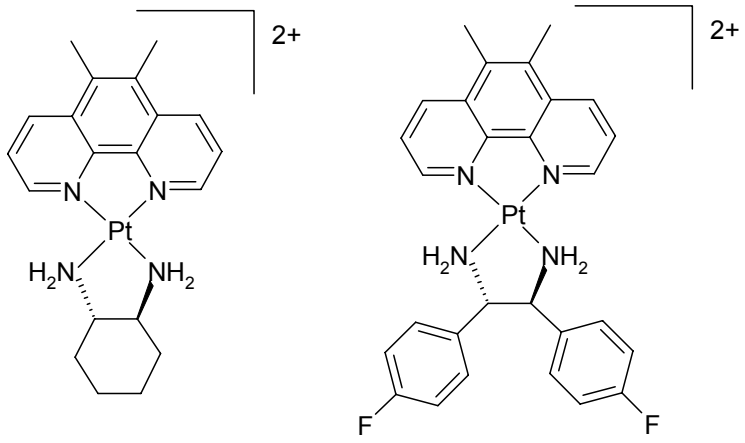
Cationic platinum(II)-1,10-phenanthrolines are a new class of cytotoxic DNA intercalator complexes.1,2 We have examined a series of our new intercalator complexes for antiproliferative activity in a panel of six human cancer cell lines. Complexes [(5,6-dimethyl-1,10-phenanthroline)(1S,2S-diaminocyclohexane)platinum(II)]2+ (56MESS), [(5-methyl-1,10-phenanthroline)(1S,2S-diaminocyclohexane)-platinum(II)]2+ (5MESS) [(4,7-dimethyl-1,10-phenanthroline)(1S,2S-diaminocyclohexane)platinum(II)]2+ (47MESS), and [(3,4,7,8-tetramethyl-1,10-phenanthroline)(1S,2S-diaminocyclohexane)-platinum(II)]2+ (3478MESS) all showed IC50 values below 0.10 μM in most cell lines tested, with the most active complex 56MESS having IC50 values even below 50 nM in some of the cell lines. These complexes were significantly more active than cisplatin and oxaliplatin, and they are the most potent Pt(II)-metallo-intercalators tested to date. On the other hand, complexes with diphenylethylenediamine instead of 1,2-diaminocyclohexane as the ancillary ligand, i.e., {(5,6-dimethyl-1,10-phenanthroline) [rac-1,2-bis(4-fluorophenyl) ethyleneediamine]-platinum(II)}2+ (56MEGMN), were considerably less active in the same cell lines.



56MESS 56MEGMN

1) Fisher DM, Bednarski PJ, Grünert R, Turner P, Fenton RR, Aldrich-Wright JR, ChemMedChem, 2007, 2: 488-495.
2) Kemp S, Wheate NJ, Buck DP, Nikac M, Collins JG, Aldrich-Wright JR, J. Inorg. Biochem., 2007, 101: 1049-1058.

P.05a

New anticancer-active fluorescent platinum(II) complexes and their intracellular processing

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Since the introduction of cisplatin into oncology practice, the studies dealing with molecular mechanisms of action have provided considerable and significant information about how cisplatin induces its antitumor effects. However, cellular processing of anticancer agents remains largely unknown. Several efforts have been undertaken to elucidate these mechanisms; one is the attachment of a fluorescent label to a cisplatin-derivative complex. This label allows for the observation the dynamic processing of cisplatin-like moieties in living cells and it provides an insight in the mechanism of action as recently reported (1). Another approach, also from our laboratory, is the use of fluorescent ligands for the synthesis of fluorescent platinum complexes (2).
Now new platinum(II) complexes with a fluorescent tail have been synthesized. The crystal structure of these complexes will be discussed in relation to its spectroscopic properties and its cytotoxic activity in different cell lines.

References

1. Molenaar, C., Teuben, J. M., Heetebrij, R. J., Tanke, H. J., and Reedijk, J. (2000) J. Biol. Inorg. Chem. 5, 655-665
2. Jansen, B. A. J., Wielaard, P., Kalayda, G. V., Ferrari, M., Molenaar, C., Tanke, H. J., Brouwer, J., and Reedijk, J. (2004) J. Biol. Inorg. Chem. 9, 403-413

P.06a

Novel bisplatinum complexes endowed with an improved in-vitro plasma protein binding and in-vivo efficacy on xenograft models

Natangelo M, Bastrup U, Torti L, Nicoli P, Piazzoni L, Bai A, Pardi G, Grugni M, Allievi C, De Munari S, Di Giovine S, Pezzoni G, Singer JW
Cell Therapeutics Inc. Bresso (Italy) and Seattle, WA (USA)

The development of multinuclear platinum complexes that cause double-stranded DNA breaks was hampered by high irreversible protein binding and low stability in plasma. Hence, a drug discovery program was designed to synthesize compounds with better pharmacokinetic and pharmacological features.
A series of bis(carboxylatoplatinum) compounds starting from {bis[trans(diamino)(chloro)platinum(II)]-μ-(1,16-diamino-7,10-diazahexadecane-N1, N16)} tetranitrate (CT-3610) replacing chloride ligands with alkylcarboxylates, were prepared. CT-47613 (bis-butyratePt) and CT-47609 (bis-capronatePt) emerged as the most interesting compounds. The in vitro protein binding of these compounds was evaluated in human, mouse, rat and dog plasma. In the absence of an appropriate method to investigate the percentage of the free drug, the extent of the free plus non-covalent bound drug was measured. Plasma was incubated at 37°C up to 8 h and at selected time points aliquots were analysed by LC-MS-MS, after acetonitrile protein precipitation. For each compound and specie, binding kinetics was described by estimation of the half-life in replicates. The cytotoxic effect was evaluated in human ovarian tumor cell lines A2780 and cisplatin resistant variant (A2780/CDDP) by WST assay. In vivo activity of both complexes was investigated, by iv administration, compared to platinum clinical agents on different human xenograft tumor models.
Both CT-47613 and CT-47609 showed a similar in vitro plasma protein binding profile in all the different species tested, with half-lives in the order of 2-3 hours. When compared to the corresponding parent drug CT-3610, both compounds exhibit lower affinity for plasma proteins, with a plasma half-life from about 1.5 to 5 times longer depending on the specie. In addition, preliminary data on the qualitative metabolic profile in human plasma suggest an increased stability to metabolic breakdown compared to the original compound.
The compounds were extremely potent on human ovarian tumor cell line A2780 and were partially cross-resistant with cisplatin.
Both complexes showed interesting in vivo antitumor efficacy in ovarian A2780 tumor models and in refractory platinum clinical agents tumor models such as A2780/CDDP, LX-1 (lung) and LoVo (colon). CT-47613 and CT-47609 showed remarkable activity in terms of primary Tumor Growth Inhibition and Tumor Growth Delay.
In conclusion, the improved protein-binding kinetic profile in human plasma together with the interesting antitumor efficacy makes CT-47609 and CT-47613 attractive candidates for further development.

P.07a

Studies on new trans-planaramineplatinum(II) complexes

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Currently attention is given to rule-breaker platinum compounds with the aim of widening spectrum of activity, reducing side effects and improving the mode of delivery. One such class of compounds are the trans-planaramineplatinum(II) complexes, some of which are found to be more active than cisplatin against cisplatin-resistant cancer cell lines. In this project we have prepared a number of new trans-planaramineplatinum(II) complexes of the forms: PtCl2(NH3)L and PtCl2L2 where L = 2-hydroxypyridine, 4-hydroxypyridine, 2,3-diaminopyridine and 2-methylimidazole, code named DH1, DH2, DH3, DH4 and DH5 (structures given in Figure 1). We have determined the nature of interaction of the compounds with pBR322 plasmid DNA and salmon sperm DNA and are currently in the process of quantifying their activity against a number of human ovarian cancer cell lines. The results of the study will be discussed in terms of structure-activity relationships.

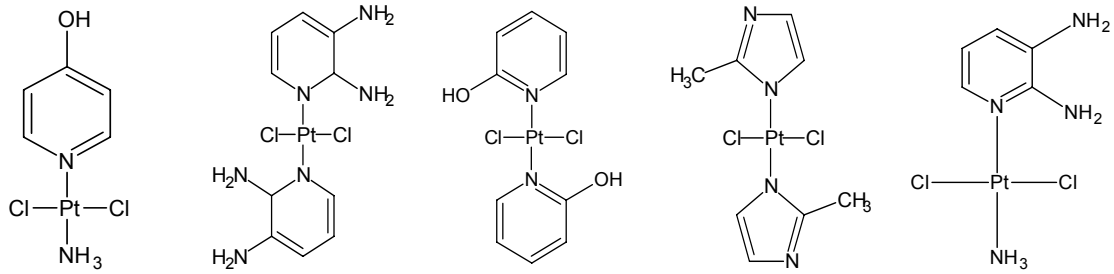

Figure 1: structure of *trans*-planaramine platinum(II) compound

P.08a

Gold(III) compounds as anticancer agents: mechanistic relevance of metal-protein interactions

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Gold(III) compounds constitute an emerging class of anticancer metallodrugs. During the past decade a conspicuous number of gold(III) complexes were reported to be sufficiently stable under physiological-like conditions and to display a relevant cytotoxic activity against selected human tumor cell lines. There is obviously large interest in understanding the precise mechanism of action of these novel cytotoxic compounds. Based on the experimental evidence collected so far we hypothesize that these novel metallodrugs, in most cases, produce their biological effects through "DNA-independent" mechanisms. In particular, strong inhibition of thioredoxin reductase and alteration of mitochondrial functions were clearly demonstrated in a few cases, that are probably the basis for the relevant proapoptotic effects. These observations led us to investigate the reactions of cytotoxic gold(III) compounds with model proteins to gain molecular-level information on the interaction modes. Valuable insight into the nature of the resulting gold(III)-protein adducts was derived from ESI MS analysis of appropriate models as exemplified here by the study of the reactions of two representative gold(III) compounds with cytochrome c and ubiquitin. The relevance of direct protein binding and damaging for the overall mechanism of action is outlined.

P.09a

Studies on the activation of oxoplatin and cellular resistance

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Oxoplatin, cis,trans,cis-[Pt(NH3)2(OH)2Cl2], is being developed in Germany in a new formulation for the oral therapy of cancer. Oxoplatin is commonly believed to be an inactive prodrug of cisplatin, which likely forms in vivo when the Pt(IV) is reduced to Pt(II) and the axial hydroxo ligands are lost. We tested the hypothesis that cancer cells are capable of activating oxoplatin to a cisplatin-like species by comparing the spectrum of cytotoxic activity of these two Pt complexes in a panel of 17 human cancer cells. With the help of regression analysis, it was found that cisplatin and oxoplatin show significantly similar activity profiles in the panel of cell lines, suggestive of similar mechanisms of action. In other experiments, four cell lines were made resistant to either cisplatin (5637 and KYSE-70) or to both oxoplatin and cisplatin (HL-60 and SISO). The cross-resistance in these cell lines to cisplatin and oxoplatin was then compared (see Table). It was found that the resistant cell lines are cross-resistant to cisplatin and oxoplatin, further evidence that oxoplatin is a prodrug for cisplatin. Gene array data from the cisplatin and oxoplatin resistant HL-60 cell lines will also be presented. Finally, oxoplatin alone is not able to bind to calf thymus DNA in vitro but cell physiological concentrations of both glutathione and ascorbic acid activate oxoplatin to platinum species that irreversibly bind to DNA. While these data indicate that oxoplatin and cisplatin maybe activated in cancer cells to the same or similar cytotoxic Pt species, other data from cell cycle specificity experiments in the Colo205 cells and gene expression studies in H526 SCLC cells indicate differences in the mechanism of cytotoxicity between cisplatin and oxoplatin.

Cell line/Drug	Cisplatin IC_{50}	RF[a] Cisplatin	Oxoplatin IC_{50}	RF[a] Oxoplatin
HL-60	0.41 ± 0.08[b]		1.21 ± 0.30	
HL-60-CDDP	3.39 ± 0.68	8.3	53.3 ± 1.5	44.1
HL-60-OXO	3.26 ± 0.33	8.0	47.8 ± 12.8	39.5
5637	0.35		4.51	
5637-CDDP	1.5	4.3	13.95	3.1
SISO	0.07 ± 0.01		0.95 ± 0.46	
SISO-CDDP	0.30 ± 0.06	4.03	2.29 ±0.45	2.40
SISO-OXO	0.22 ± 0.07	2.95	4.8 ± 2.0	5.03
KYSE-70	0.39 ± 0.05		5.45 ± 0.17	
KYSE-70-CDDP	1.13 ± 0.17	2.90	13.1 ± 3.6	2.40

a) RF = resistance factor, b) values (in µM) are averages ± SD

P.10a

Light-activation of trans-diazido-Pt(IV)-complexes to cytotoxic and DNA-binding species

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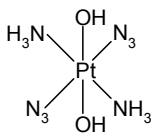
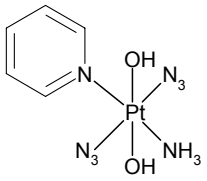
Photoactivatable chemotherapy with light-sensitive Pt(IV) complexes promises to increase the selectivity and hence the efficacy of platinum-based anticancer treatment. cis-Diazido-Pt(IV) complexes, such as cis,cis,trans-[Pt(NH3)2(N3)2(OH)2], possess favorable chemical and biological properties for light activation.1-3 We have been studying the effects of light on the cytotoxic effects of two new trans-diazido-Pt(IV)-complexes (FM137 and FM165) in cultures of human cancer cell lines. In addition, biochemical studies are underway to understand the mechanisms of action of these novel complexes. Here, we report the cytotoxic potency of FM137 and FM165 in two human bladder cancer cell lines (5637 and RT-4), two human lung cancer cell lines (A427 and LCLC-103H) a human pancreas cancer cell line (DAN-G) and a human breast cancer cell line (MCF-7). The ability of the complexes to inhibit the growth of cancer cells, with and without irradiation with light of wavelength 366 nm, was studied. Light dramatically increased the cytotoxicity of the complexes. It was found that FM165 is considerably more active than FM137 when both complexes were irradiated with light of the same intensity. The IC50 values for complex FM165 range between 20 and 80 μM in the six cell lines, with the order of sensitivity decreasing as follows: 5637 = LCLC-103H > A427 = DAN-G > MCF-7 = RT-4. Thus, there appear to be cell lines that are more sensitive and others that are less sensitive to the cytotoxic effects of photoactivated FM165. Light induced irreversible binding of Pt to calf thymus DNA was investigated by AAS. Both complexes lead to similar levels of DNA-platination 24 h following irradiation with uv light but the rates of platination over the first few hours after irradiation are much faster for FM137 than for FM165. Effects of added chloride, glutathione and ascorbic acid on the rates of DNA platination will also be described.



FM 137



FM 165

1) Bednarski, P.J:, Grünert, R., Zielzki, M., Wellner, A., Mackay, F.S., Sadler, P.J. *Chem. Biol.,* 2006, **13**: 61-67.
2) Mackay, F.S., Woods, J.A., Moseley, H., Ferguson, J., Dawson, A., Sadler, P.J. *Chem. Europ. J.,* 2006, **12**: 3155-3162.
3) Bednarski, P.J., Mackay, F.S., Sadler, P.J. *Anti-Cancer Agents Med Chem,* 2007, **7**:75-93.

P.11a

Cytotoxicity, mutagenicity, cellular uptake, DNA and glutathione interactions of lipophilic trans-platinum complexes tethered to 1 adamantylamine

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Cytotoxicity and mutagenicity of trans,trans,trans-[$PtCl_2(CH_3COO)_2(NH_3)$(1-adamantylamine)] [trans-adamplatin(IV)] and its reduced analog trans-[PtCl2(NH3)(1-adamantylamine)] [trans-adamplatin(II)] were examined. In addition, the several factors underlying biological effects of these trans-platinum compounds using various biochemical methods were investigated. A notable feature of the growth inhibition studies was the remarkable circumvention of both acquired and intrinsic cisplatin resistance by the two lipophilic trans-compounds. Interestingly, trans-adamplatin(IV) was considerably less mutagenic than cisplatin. Consistent with the lipophilic character of trans-adamplatin complexes, their total accumulation in A2780 cells was considerably greater than that of cisplatin. Inert platinum(IV) complexes must be reduced to their more reactive platinum(II) analogs to exert anticancer activity. The results also demonstrate that trans-adamplatin(II) exhibits DNA binding mode markedly different from that of ineffective transplatin. In addition, the reduced deactivation of trans-adamplatin(II) by glutathione seems to be an important determinant of the cytotoxic effects of the complexes tested in the present work. The factors associated with cytotoxic and mutagenic effects of trans-adamplatin complexes in tumor cell lines examined in the present work are likely to play a significant role in the overall overall antitumor activity of these complexes.

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P.12a

Using fluorescent tagging to monitor the cellular fate of drugs delivered by cobalt chaperones

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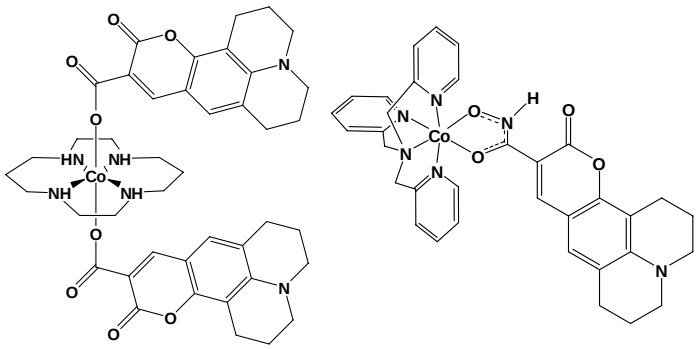
The selective activation of prodrugs in tumour environments is one of the most promising approaches for minimising the toxicity of anticancer drugs. However, the rational design of such prodrugs requires an intimate knowledge of their localised distribution and activation in the tumour and this has been lacking to date. Cobalt(III) complexes are capable of acting as chaperones for antitumour agents, selectively delivering them to hypoxic tumour environments. This is due to their having a stable cobalt(III) oxidation state which is selectively reduced to a labile cobalt(II) state in hypoxic environments. The labile cobalt(II) complex subsequently undergoes fast aquation, releasing its original ligands, and hence the bound cytotoxins. Previous studies have employed techniques including XANES, and fluorescence tagging to investigate aspects of these processes.

Fluorescence tagging has proven to be an effective means for monitoring the cellular uptake, localisation, and mechanisms of action of these agents, as cobalt is able to alter the fluorescence of bound fluorophores. This has allowed us to monitor the coordination and oxidation state of the complex in cellular environments. Using changes in fluorescence, we have been able to visualise differences in uptake and distribution of the cobalt complexes and their fluorescent ligands in A2780 ovarian cancer cells. We found that different ancillary ligands and fluorophore chelating groups altered the uptake and distribution of these complexes. The cobalt complex having a coordinated carboxylate fluorophore, A, showed distinct nuclear localisation while another complex with a hydroxamate fluorophore, B, or their respective free fluorophores, did not. The altered distribution of the complexes correlated with altered cytotoxicity, as the complex that displayed nuclear localisation was also observed to be the most cytotoxic. This is thought to be a consequence of the complex acting as a delivery system for the free fluorophore, which is only able to exert cytotoxicity when it enters the nucleus. These results suggest that changing the coordination environment of such complexes may allow targeted distribution of the complexes in cells and therefore may be important in affecting cytotoxicity.



A **B**

P.13a

Pharmacological modulation of tumor-released microvesicles: a tool for rescuing immune suppression in cancer patients?

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Human tumors release vesicular structures, generally defined as microvesicles (MV), which have been recently found to display a broad array of detrimental effects on the immune system. Through the expression of bioactive FasL and TRAIL molecules, MV induce apoptosis in activated anti-tumor T cells, while through molecular pathways that are presently under investigation, they promote the generation of myeloid suppressive cells exerting TGFß-mediated inhibition of T cell proliferation and function. The evidence that immunosuppressive MV of tumor origin can be found in abundance in tumor lesions and sera from cancer patients suggests a potential role of this pathway in in vivo tumor progression. Pharmacological strategies aimed at negatively modulating this process could thus interrupt MV-triggered suppressive pathways and ameliorate tumor/immune system interactions. The mechanisms regulating MV release by cancer cells are not fully characterized yet, although cytoskeleton structures, including microtubules and actin-filaments, are likely involved, as suggested by the significant decrease of MV shedding observed after treatment with cytochalasin D, latrunculin A, nocodazole or interfering with ezrin expression. Chemotherapeutic agents acting on microtubule polymerization, such as paclitaxel and vinblastin, exhibit inhibitory effects on MV release, together with drugs blocking V-ATPase pump activity. In contrast, non-cytotoxic concentrations of the DNA damaging agent cisplatin, appear to exert opposite effects and enhance MV shedding. Interestingly, the reduction of MV release by tumor cells is associated with a more efficient recognition by antigen-specific cytotoxic T cells. These data support the hypothesis that immune recognition of tumor cells can be improved not only by increasing target immunogenicity, but also by interfering with suppressive mechanisms through pharmacological intervention.

P.14a

Photoactivated platinum anticancer complexes

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Despite the widespread use of platinum-based drugs in cancer chemotherapy, the occurrence of side-effects and resistance remains a major limitation. We are investigating novel diazido Pt(IV) complexes as photoactivatable prodrugs. This offers the possibility of site-specific activation; such targeted therapy may help to alleviate the side-effects associated with existing platinum-based agents. Upon irradiation the diazido Pt(IV) complexes are reduced to reactive Pt(II) species, and are phototoxic to a range of cancer cell lines. The DNA platination profile of these species is similar to that of cisplatin; however cell morphology experiments show interesting effects indicative of a different mechanism of action (1).

We are investigating diazido Pt(IV) complexes with reactive functional groups to allow for labelling and targeting. The functional group may provide a site for attachment of biologically-active species, a strategy which is becoming increasingly favoured in drug design, and has been used in the synthesis of a number of novel platinum complexes (2). We report here the preparation and properties of diazido Pt(IV) complexes containing chelated diamine ligands with hydroxyl side chains.

We thank the EPSRC, the University of Edinburgh and the University of Warwick (studentship for SJF) and Scottish Enterprise for support, and Dr Julie Woods (Dundee) and COST D39 for discussions.

1. P.J. Bednarski, R. Grünert, M. Zielzki, A. Wellner, F.S. Mackay and P.J. Sadler, Chemistry & Biology, 2006, 13, 61
2. E. Wong and C.M. Giandomenico, Chem. Rev., 1999, 99, 2451

P.15a

Structure-transport and structure-activity relationships of oxaliplatin analogues with various leaving groups

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The use of platinum complexes in cancer chemotherapy is often limited by inherent or acquired resistance. To overcome the resistance several chemical modifications of the platinum complexes have been suggested. Our work aims to analyze the influence of the leaving group in platinum complexes on their cellular accumulation and cytotoxic activity. For this purpose we chose six complexes each with a 1R,2R-diaminocyclohexane (DACH) carrier ligand and various leaving groups: 1R,2R-DACH-cyclobutane-1,1-dicarboxylatoplatinum(II), 1R,2R-DACH-dichloro-platinum(II), 1R,2R-DACH-malonatoplatinum(II), 1R,2R-DACH-oxalatoplatinum(II), 1R,2R-DACH-dihydroxooxalatoplatinum(IV), 1R,2R-DACH-bis(3-carboxypropanoato) oxalatoplatinum(IV). The reactivity towards nucleotides and the lipophilicity (log P) were determined. Subsequently, we investigated the intracellular platinum concentrations and the cytotoxic activity after incubation with the complexes in the ileum carcinoma cell line HCT-8 and their oxaliplatin-resistant variant HCT-8ox.
The reactivity of the complexes towards dGMP was studied using capillary electrophoresis. The log P values were determined by the shake-flask method. For uptake studies 2x106 cells were incubated with each platinum complex (100 µM) up to 2h. Intracellular platinum concentrations were measured by flameless atomic absorption spectrometry (FAAS). MTT assays were performed to estimate cytotoxicity (EC50 values).
Determination of the reactivity revealed the following order: chloro > oxalato > malonato > cyclobutanedicarboxylato > dihydroxooxalato = bis(3-carboxypropanoato)oxalato. The ranking of the complexes in terms of lipophilicity was: cyclobutanedicarboxylato > chloro > malonato > oxalato > dihydroxooxalato > bis(3-carboxypropanoato)oxalato.
More lipophilic complexes were found to accumulate in the cells to a higher extent in the early uptake phase, indicating passive diffusion as a main uptake mechanism in the first 10 minutes. Linear regression analysis was performed to assess the rate of uptake. The slopes did not correlate with the log P values, but were reduced in the resistant cell line. These data suggest that lipophilicity is not the rate-determining factor for the uptake of oxaliplatin analogues. However, increasing reactivity led to enhanced intracellular platinum concentrations and higher cytotoxic activity.
In conclusion, the early uptake phase is mainly determined by passive diffusion. Subsequently, other mechanisms, e.g. active transport, seem to be responsible for cellular uptake. The reactivity of the complexes may govern the later uptake rate. The increased cytotoxicity of the more reactive complexes may be a consequence of the elevated uptake and/ or a more effective reaction with the DNA.

P.16a

Platinum-anthraquinone complexes: best of both worlds?

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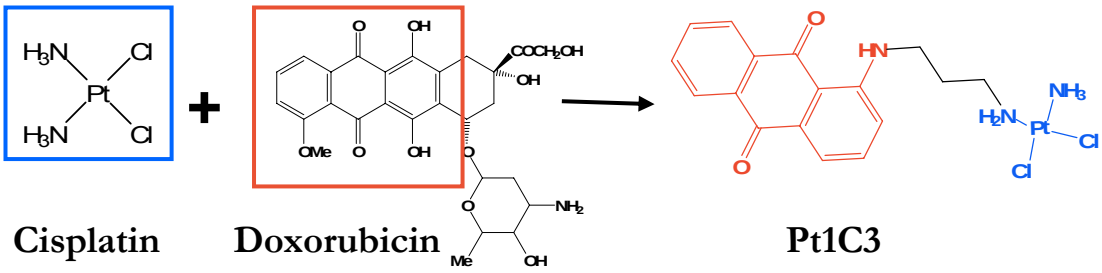
Platinum(II) complexes and anthracyclines, despite their clinical limitations, remain two of the most widely used classes of chemotherapeutic agents. Efforts to develop secondary and tertiary agents with improved drug efficacy have resulted in the synthesis of such species as the hybrid platinum-anthraquinone complexes. The rationale behind the tethering of platinum moieties with intercalating anthracycline components is to combine desirable properties from both its parent analogues, including high lipophilicity, DNA selectivity, and intrinsic fluorescence. In vitro studies of these hybrid complexes have indeed found them to possess a combination of interesting biological properties from both of their parent analogues, including rapid cellular and nuclear uptake and novel modes of action. Interestingly, confocal micrscopy studies of these complexes have revealed drug fluorescence to be localised within lysosomes, and not its intended site of action, the nucleus.
Here we report our investigations into the cellular behaviour of one platinum-anthraquinone complex, Pt-1C3. We have employed a wide range of techniques, including confocal microscopy, sub-cellular fractionation and cytotoxicity assays, as well as, employing some novel methods, such as the use of pathway inhibitors and EXAFS, to elucidate the intracellular interactions of these complexes.



P.17a

Steroids as delivery vectors for non-conventional Pt(II) metallodrugs

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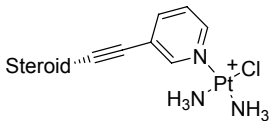
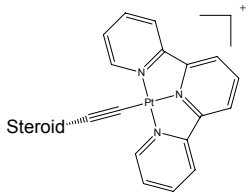
Sex hormones such as estrogens and testosterone have been recently identified as important factors in the development and treatment of cancers of the feminine and masculine reproductive system. Many such cancers show over-expression of the receptors for these hormones. We therefore postulated that the elevated levels of steroids receptors detected in many cancers of the breast, ovary, uterus and prostate might afford a way of targeting these tissues. In background work, conjugates of metals and bioactive steroids have been created, focused on the estradiol receptor(ER) [1,2]. To the best of our knowledge no steroidal metal complexes have targeted the androgen receptor (AR) with the aim of localising cytotoxic drugs. We describe such agents herein[3].

Non-conventional Pt(II) complexes had been attached to testosterone and estradiol derivatives. Different designs with and without the presence of leaving groups have been used, aiming to different binding to DNA.(Fig. 1)





a) Leaving group design b) Non-leaving group design

Fig.1

Activity against cancer cell lines showing different spectra of expression of estrogen (ER) and androgen receptors(AR), and interaction with DNA of these compounds have been studied, showing dramatic results.

P.18a

Oxaliplatin accumulation and DNA adduct formation in oxaliplatin resistant colorectal and ovarian cancer cell lines: relation with mRNA expression of transport and DNA repair genes

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Resistance towards oxaliplatin has been associated with decreased uptake and formation of platinum DNA adducts and enhanced repair of the platinum DNA adducts. Several transporters have been described that might mediate the uptake and efflux of oxaliplatin like the human copper transporter hCTR1 and the ATPases ATP7A and ATP7B. The organic cation transporters OCT1, OCT2 and OCT3 might be involved in the uptake of platinum compounds. In order to study whether these these transporters play a role in oxaliplatin resistance we determined the mRNA expression of these transporters in resistant variants of the colorectal and ovarian cancer cell lines LoVo-92 (wt-p53), LoVo-Li (non functional p53) and A2780 (wt-p53) respectively. Two resistant variants of each cell line were tested, the first was made resistant with 4 hour pulses (/4OHP), to better mimic clinical exposure, and the second was with continuous exposure (/cOHP) to oxaliplatin. The mRNA expression of the transporters and also ERCC1 in untreated cells was related to the total accumulation of oxaliplatin and the formation of platinum DNA adducts. Cells were exposed for 24 hours ± 3 hours drug free period at a concentration of 200 μM. LoVo-92 showed a total oxaliplatin accumulation of 767 pmol/106 cells. In LoVo-92/4OHP and LoVo-92/cOHP the accumulation was 40% compared to the parental and accompanied by reduced DNA adduct formation to 25% and 16%. Expression of the uptake transporters hCTR1, OCT1, OCT2 and OCT3 was decreased to 10-80% while ATP7A and ATP7B were increased to 120%. ERCC1 was about 85% of the parental expression. In LoVo-Li the accumulation was 226 pmol/106 cells. Accumulation was decreased to 72% in LoVo-Li/4OHP and 91% in LoVo-Li/cOHP while a reduction to 75% in adduct formation, LoVo-Li/4OHP, but an increase to 165% in LoVo-Li/cOHP was observed. LoVo-Li/4OHP showed decreased expression of the in- and efflux transporters to 20-60%. In LoVo-Li/cOHP uptake transporters, except OCT1, and the efflux transporters were decreased to 5 - 80%. No change in ERCC1 expression was observed. A2780 showed an accumulation of 523 pmol/106 cells and was similar in A2780/4OHP but 50% in A2780/cOHP compared to parental cells. However DNA adduct formation was increased to 153% in A2780/4OHP but similar in A2780/cOHP. OCT1 was decreased to 50% and 25% in A2780/4OHP and A2780/cOHP, respectively. OCT2 and OCT3 were not detectable and hCTR1 showed an increase in expression but was not detectable in the parental cell line. ATP7A was reduced to 34% and 47% and ATP7B to 21% and 16% in A2780/4OHP and A2780/cOHP respectively. Also ERCC1 showed a decreased expression to 6% and 14%. In conclusion: resistance was significantly correlated with accumulation of oxaliplatin and was mainly accompanied with decreased expression of uptake transporters and was highest for OCT3.

P.19a

Oxoplatin – an oral platinum(iv) compound evaluated in comparison to cisplatin in vitro.

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Oxoplatin (cis-diammine-dichloro-trans-dihydroxo-platinum(IV)) is currently being developed as an oral anticancer agent. The antitumor activity of oxoplatin was screened in vitro using MTT proliferation assays in a panel of 33 human cancer cell lines. Oxoplatin induced growth inhibition, generation of reactive oxygen species (ROS) as detected by dihydroethidium fluorescence in flow cytometry, and triggered cell death as revealed by annexinV/propidium iodide double staining in various cell lines. Mean IC50 values obtained for oxoplatin were approximately 2.5-fold higher compared to cisplatin. In contrast to cisplatin, oxoplatin was not significantly inactivated by 400µM uric acid in vitro, and following a 15 min exposure of oxoplatin to 0.1N HCl representing gastric acidity, the resulting platinum species revealed a more than twofold increase in cytotoxicity. Similar results for HCl-pretreatment were obtained for JM 149 (ammine-dichloro-(cyclohexylamine)-dihydroxo-platinum(IV)), however, not for the parent drug JM 216 / satraplatin (bis-acetato-ammine-dichloro-cyclohexylamine-platinum(IV)) and trans-oxoplatin (all-trans-diammine-dichloro-dihydroxo-platinum(IV)). Cell cycle perturbations induced by different platinum compounds in COLO 205 colon cancer cells varied (% alteration compared to medium controls): cisplatin - arrest in G2M (+ 70%); oxoplatin – accumulation in S (+ 35%), HCl-pretreated oxoplatin – accumulation in S (+ 25%) as well as G2M (+ 45%), and satraplatin – arrest in S (+ 55%) as well as G2M phase (+ 15%). Activation of oxoplatin by ascorbic acid in vitro resulted in cytotoxic activity comparable to that of cisplatin. Using the small cell lung cancer (SCLC) cell line H526 exhibiting similar sensitivity to oxoplatin and cisplatin, genome wide expression profiling (Applied Biosystems Human Genome Survey Microarray V2.0) was performed following application of the two compounds. While the highest upregulated genes observed in cisplatin-treated cells included components of the cytoskeleton and associated proteins, cells treated with oxoplatin revealed increased expression of a subgroup of ribosomal proteins as well as downregulation of cytoskeletal proteins. Only 35% of the downregulated genes and 10% of the upregulated genes were identical for cisplatin and oxoplatin treatment, pointing to differences in molecular mechanisms of action of these two platinum compounds despite an observed crossresistance in resistant cell lines. In conclusion, oxoplatin constitutes a potent oral agent that may be either transformed to more active compounds by gastric acid or acidic tumor regions in dependence of the respective pharmaceutical formulation and by intracellular reduction.

P.20a

Does cisplatin interfere with telomeric DNA?

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Telomeres are DNA-protein structures capping chromosome ends to protect them from degradation and end-to-end fusions. Telomeric DNA consists of highly repetitive short sequences of guanines residues (TTAGGG)n, followed by a single-stranded DNA overhang at the 3' extremity. Alterations of the telomeres, either due to the reduction of their total length, or to the reduction of their 3' overhang length or to the dissociation of the telomeric proteins (TRF1, TRF2 and Pot1), are leading to the death of the cells. As platinum complexes bind preferentially to guanines, telomeric DNA is a potential target. We have shown that cisplatin is able (i) to cross-link the 3'overhang telomeric DNA folded as a G-quadruplex structure (1,2) and (ii) to prevent the binding of TRF1 and TRF2 to the double-stranded telomeric DNA. Brought together, these data suggest that the platination of telomeric DNA could interfere with the telomere integrity. The effect of cisplatin treatment on telomere length of different cell lines will be discussed.

References

(1) Redon, S., Bombard, S., Elizondo-Riojas, M. A. and Chottard, J. C. (2003).Nucleic Acids Res., 31, 1605-1613
(2) Ourliac-Garnier, I and Bombard, S (2007) GG sequence of DNA and the human telomeric sequence react with cis-diammine-diaquaplatinum at comparable rates. J. Inorg. Biochem. 101, 514-524

P.21a

Targeting nucleotide excision repair as a mechanism to increase cisplatin efficacy

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The main repair pathway that cells employ to repair cisplatin damage is the nucleotide excision repair (NER) pathway. Recognition of DNA damage is the rate limiting step in this pathway and involves binding of a recognition complex that includes Replication Protein A (RPA) and Xeroderma Pigmentosum Group A (XPA) to the cisplatin-DNA damage site. By targeting and inhibiting recognition, repair of the DNA damage will be reduced and cisplatin adduct levels and duration will be increased. To identify small molecule inhibitors (SMI) of RPA and XPA we developed a high-throughput screening assay using fluorescence polarization (FP). A fluorescent labeled DNA along with purified RPA and XPA were employed in a 384 well format and used to screen the ChemDiv library of chemical compounds. We have screened over 40,000 compounds and identified a series of SMIs for XPA and for RPA. We have analyzed these hits in secondary screens using FP and electrophoretic mobility shift assays, confirming the activity of a subset of both the XPA and RPA hits. Cellular activity of a lead RPA inhibitor was assessed in human lung cancer and ovarian cancer cell line using a series of viability, cell cycle and apoptosis assays. An antiproliferative effects was observed in viability assays and flow cytometric analysis reveal a prolongation of S-phase, consistent with inhibition of RPA's role in DNA replication. Flow cytometry was also employed to measure apoptosis using propidium iodide and annexin V staining. The RPA SMI was found to include apoptosis in both a time and concentrations dependent fashion in both ovarian and lung cancer cell lines. Preliminary analysis in NOD SCID mice reveal minimal toxicity and position us to assess the efficacy of this compound in a lung tumor xenograft model in conjunction with platinum based therapy.

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P.22a

Influence of DNA structure and orientating on activation of DNA-PK

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Cisplatin is a widely used chemotherapeutic DNA damaging agent that is often used in conjunction with ionizing radiation (IR). IR induced DNA double strand breaks are repaired by the non-homologous end joining (NHEJ) pathway and are lethal if not repaired. The molecular mechanism behind cisplatin-IR treatment has revealed that cells with active NHEJ catalyzed repair of DNA double strand breaks (DSB) exhibit a synergistic interaction while cells deficient in NHEJ do not. The molecular mechanism responsible for the synergistic effect of cisplatin and IR has revealed that a cisplatin lesion near the terminus of a DNA double strand break results in obstruction of NHEJ catalyzed repair. Thus, after a DSB is induced by IR, the NHEJ pathway is required to repair the break, but this repair process is stalled by the bulky cisplatin lesion at the DNA terminus. While this data suggests an important role for NHEJ in the mechanism of cisplatin and IR efficacy, the specifics of the NHEJ pathway are not fully characterized. The first step in NHEJ is the recruitment and activation of the DNA-dependent protein kinase (DNA-PK). DNA-PK is activated once the kinase is bound to the DSB, and this activity is required to signal to other downstream components in the pathway. Understanding the mechanism of DNA-PK activation and the role of terminal nucleotide structures will allow us to elucidate the role of cisplatin-DNA-lesions in cisplatin dependent radiosensitization. We have investigated the effect of a variety of DNA substrates on DNA-PK activation in an attempt to understand this molecular mechanism. Results demonstrate that, unlike the increase in activation seen with DNA-PKcs, heterotrimeric DNA-PK is not preferentially activated by double strand DNA substrates with single strand overhangs, nor by Y-shaped DNA substrates. Furthermore, we show that DNA-PK activation, in comparison to substrates with a 5' single strand overhang, is inhibited by substrates containing a 3' overhang. Additional data demonstrate enhanced DNA-PK activation via dimer formation of two holo-enzyme complexes. The presence of a cisplatin lesion on a single strand overhang may then be responsible for decreasing this DNA-PK activity, thus leading to overall decreased repair of DNA DSB. These results suggest that the position of cisplatin lesions in the context of a DNA DSB will impact NHEJ efficiency.

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P.23a

X-ray crystal structure of a cytotoxic monofunctional platinum–DNA adduct, {Pt(NH3)2(pyridine)}2+ bound to deoxyguanosine in a dodecamer duplex

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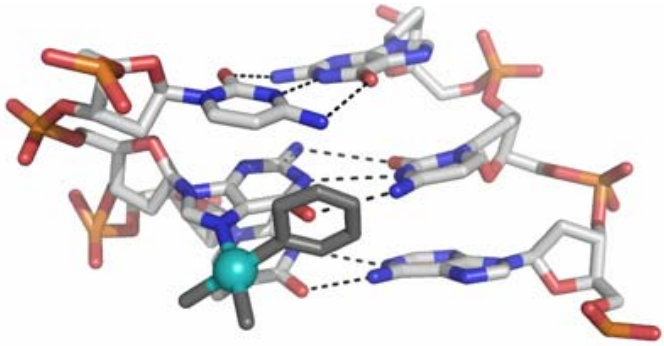
The cationic complex [Pt(NH3)2(pyridine)Cl]+ displays antineoplastic activity in animal tumor models despite violating the classical structure-activity relationship established for platinum antitumor agents.1 This complex binds to DNA in a monodentate fashion at the N7 position of guanine residues, yet it blocks the action of DNA polymerases in a manner that is unique to monofunctional platinum compounds.2 Moreover, recent evidence has demonstrated that this complex is taken up by cells via organic cation tranporters, a family of membrane-spanning proteins expressed in colorectal cancer cells, which presents an opportunity for selective delivery to the tumor.3 Here we present the 2.17 Å resolution X-ray crystal structure determination of an monofunctional dG adduct of this complex on a DNA duplex with a sequence similar to that of previously reported platinated DNA structures. The global structure of the DNA is quite different from that of DNA containing a platinum intrastrand d(GpG) cross-link. The latter platinated duplex is bent by ~40° towards the major groove at the site of the cross-link; however, the monofunctional platinum–dG lesion causes no significant distortion of the double helix. The structural features of this model are discussed with respect to the potential of [Pt(NH3)2(pyridine)Cl]+ and related compounds to serve as a new platform for platinum anticancer drug design. This work was supported by a grant from the National Cancer Institute.
This work was supported by NIH grant CA82741 and funding from the Flight Attendants Medical Research Institute and the Walther Cancer Institute.



References
1. Hollis LS; Amundsen AR; Stern EW *J. Med. Chem.* **1989,** 32, 128-136.
2. Hollis LS; Sundquist WI; Burstyn, JN; Heiger-Bernays WJ; Bellon SF; Ahmed KJ; Amundsen AR; Stern EW; Lippard SJ *Cancer Res*. **1991**, 51, 1866-1875
3. Lovejoy KS; Lippard SJ **2006**, unpublished results.

P.24a

Identification of determinants of cisplatin response in fission yeast using genome-wide deletion mutant

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Genetic alterations contributing to drug response and resistance to cisplatin have not been fully defined. Given the powerful molecular tools now available, the combination of molecular pharmacology and molecular biology expertise in studying model organisms could lead to a rapid progress in the discovery of strategies to overcome cisplatin resistance. Whereas Saccharomyces cerevisiae has been already employed extensively, the use of the fission yeast Schizosaccharomices pombe in pharmacology studies is still poor, in spite of the availability of the complete genome sequence and of additional tools including microarray platforms. Using conventional approaches and global genome expression analysis, we have previously identified pathways relevant for cisplatin response in fission yeast. In particular, cisplatin activates a stress response including gluthathione-S-transferase, heat shock, proteasome and DNA repair genes (Gatti et al., 2004, Cell. Mol. Life Sci.). However conclusive findings about the specific contribution of different pathways to cisplatin response have been limited in such organism by the analysis of small sets of mutants.
Thus, the present study was designed to clarify the contribution of single genes to response to cisplatin. Here, we describe our preliminary results aimed at providing the proof of principle of the involvement of specific genes modulated by cisplatin treatment in cell response to the drug. Sensitivity to cisplatin in a panel of non essential haploid deletion mutants, belonging to a collection of haploid strains constructed through homologous recombination in S. pombe, is being examined. For this purpose we use a microtiter plate assay to compare sensitivity of deletion mutants to that of the wild-type strain. Genome-wide deletion mutant library was constructed in large scale by PCR-based targeted mutagenesis based on the S. pombe genome sequence information provided by the public website at the Wellcome Trust Sanger Institute (www.sanger.ac.uk). All deletion cassettes for mutant construction were made to contain the antibiotic-resistant gene as a selection marker, tag sequence to identify each mutant and universal sequence to amplify the tag sequences. As a selection marker KanMX4 module, that give G418 resistance to tranformants, was used (http://pombe.bioneer.co.kr). This approach is expected to permit definition of genes conferring sensitivity/resistance to cisplatin in fission yeast.

P.25a

Chemistry and biology of the phosphate clamp. New polynuclear platinum anti-cancer drug candidates

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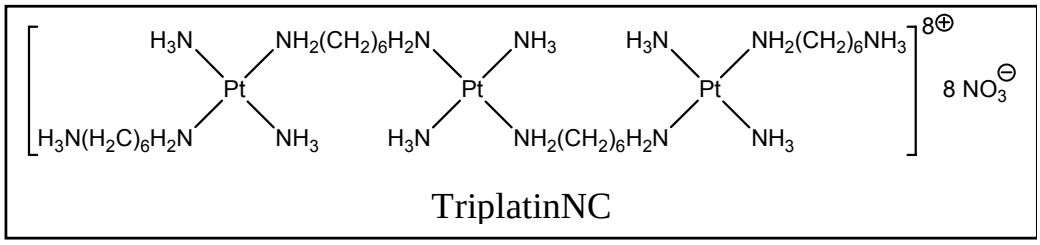
"Non-covalent" polynuclear platinum compounds (PPNC), such as TriplatinNC are direct structural analogs of the Phase II drug BBR3464, where Cl- is replaced by NH2(CH2)6NH2. TriplatinNC is of interest because it does not contain substitution-labile ligands and displays enhanced stability to metabolic deactivation, and is a further advance from the original structure-activity paradigm of platinum drugs.
TriplatinNC shows a new mode of DNA binding, the phosphate clamp, typified by interaction with the backbone and spanning the groove of DNA [1]. The melting temperature of DNA increased 15-18 oC due to the minor groove and the backbone interactions and strong hydrogen bonding between DNA and PPNC. This contribution firstly summarizes the biophysical properties of phosphate clamps in solution. Secondly, the biology is summarized. TriplatinNC showed very interesting results in enhancement of cellular uptake and high cytotoxicity in human tumor cells. Cytotoxicity is significantly less dependent on p53 status. Unlike cisplatin, oxaliplatin, and BBR3464, TriplatinNC was able to circumvent deactivation by glutathione since its cytotoxicity was not affected by inhibition of glutathione synthesis. The study illustrates fundamental differences in the mechanisms of action between "noncovalent" binding drugs like TriplatinNC and structurally related covalent binding drugs such as cisplatin, oxaliplatin, and BBR3464. TriplatinNC representsa n interesting drug for development.



TriplatinNC

[1] S. Komeda et al., *J. AM. CHEM. SOC.* **2006**, 128, 16092-16103.

P.26a

Comparative study of the interaction of the Pt(II), Pd(II) and Au(III) complexes with a thiolate-bridged zinc chelate: insight on the metal exchange mechanism in zinc finger model

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Interactions between thiolate-bridged zinc chelates and platinum complexes have been studied as models for possible in vivo interactions between platinum anticancer agents and zinc finger proteins1,2. Specifically the C2H2 zinc finger consists of a compact globular structure that contains two ß sheet and an α helix held together by a central Zn2+ ion3. Soldatovic et al.4 have demonstrated that nucleophiles like sulfur donors (hyldithiocarbamate and thiosulfate) can substitute guanosine from the complex [Pt(terpy)(N7-guanosine)]2+. In order to provide precedents for the possible interactions of platinum DNA adducts with Zinc Finger proteins, the complexes [M(dien)Cl]Clx (M=Pt,Pd/x=1; M=Au/x=2; dien = diethylenetriamine) and [M(terpy)Cl]Cl (M=Pt,Pd/x=1; M=Au/x=2; terpy = 2,2': 6',2"-terpyridine) were exposed to the Zn dithiolate, N, N'-bis(2-mercaptoethyl)-1,4-diazacycloheptanezinc(II) dimer, [Zn(bme-dach)]2, and the products defined by Electrospray Ionization Mass Spectrometry (ESI-MS), x-ray Crystallography and 195Pt-NMR spectroscopy. The presence of a leaving chloride in Pt(II), Pd(II) and Au(III) complexes facilitates electrophilic substitution involving sulfur-containing zinc finger synthetic models or, as in previous studies1, zinc finger peptidic sequences2. Following our studies1 on [Zn(bme-dach)]2 reactivity with the model compounds [M(dien)Cl]Clx (M=Pt(II),Pd(II)/x=1; M=Au(III)/x=2, we used ESI-MS to monitor the chemical species resulting from different incubation times and stoichiometric ratios M : Zn (1M:1Zn and 2M:1Zn). Monitored via ESI-MS, the reactions with Pt and Pd yielded evidence for Zn-(μ-SR)-M (M=Pt,Pd) bridges followed by zinc ejection from the N2S2 coordination sphere and subsequent formation of a trimetallic Zn-(μ-SR)2-Pt-(μ-SR)2-Zn bridged species. In the case of the [Pt(terpy)Cl]Cl reaction with the [Zn(bme-dach)]2, ESI-MS analysis suggests metal exchange by formation of [Zn(terpy)Cl]+, while the [Pt(dien)Cl]Cl reaction does not yield the corresponding [Zn(dien)Cl]+ ion. Also, ESI-MS full scan (positive mode) of the reaction between [AuCl(L)]Cl2 (L=terpy or dien) and [Zn(bme-dach)]2, recorded after mixing, have shown a rapid displacement of Zn with formation of [Zn(L)Cl]+ (L= terpy or dien) with insertion of Au into the chelate. Direct synthesis of the Zn-Pt or Zn-Pd thiolate bridged species and the Pt(N2S2) has permitted the isolation of x-ray quality crystals to confirm such bridging and metal exchanged structures. The ESI-MS, 195Pt NMR spectroscopy, and molecular structures of the di- and trinuclear complexes have provided insight into the metal exchange mechanism.

References

1. Liu, Q., Golden, M., Darensbourg, M. Y. and Farrell, N., Chem. Comm., 4360, 2005.
2. Anzellotti, A. I., Liu, Q., Bloemink, M.J., Scarsdale, J. N. and Farrell, N., Chemistry & Biology, 13, 1, 2006.
3. Omichinski, J., Clore, G. M., Appella, E., Sakagucchi, K. and Gronenborn, A. M., Biochemistry, 29, 9324, 1990.
4. Soldatovic, T. & Bugarcic, Z. D., J. Inorg. Bioch., 99, 1472, 2005.

P.01b

Effect of cisplatin (C) on prolonged infusion (PI) of gemcitabine (G) in patients with advanced non-small cell lung cancer (NSCLC): preliminary results of plasmatic pharmacokinetic (PK) and intracellular determination study

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Background. G and C combination is a common treatment in advanced NSCLC. G activity is mediated by intracellular concentration of di- and triphosphate derivates (dFdCTP), which is dose dependent. High plasma levels of G are achieved by PI, holding a fixed dose rate (FDR) of 10 mg/m^2/min. In a previous PK study, with G 600 to 1200 mg/m^2, at FDR of 10 mg/m^2/min, the target 15μM plasma concentration was achieved in 70% of pts at 600-900 mg/m^2, but only in 44,4% at 1000-1200 mg/m^2. Objective response rate was 56.5% vs 46.1% among pts receiving < 1000 or ≥ 1000 mg/m^2 respectively [Lung Cancer 2003; 41(S2):230]. Based on these findings, we have measured both plasma and intracellular concentration of G, and its metabolites, at the doses of 600 and 1200 mg/m^2, PI, aimed to evaluate the effect of C administration on the G plasmatic PK and the intracellular concentration. **Methods**. G was administered on days 1 and 8, by an electronic pump, at a FDR of 10 mg/m^2/min. C 75 mg/m^2 was administered on day 8. Four pts received G at 600 mg/m^2 during the first and 1200 mg/m^2 during the subsequent cycles. Four pts received 1200 mg/m^2 during all treatment. For PK evaluation, 8 blood samples were collected at specific intervals (from 0 to 24 hours) on the days 1 and 8 of the first two chemotherapy courses, for a total of 32 samples per patient. G and its metabolites levels were determined by a validated HPLC-tandem mass spectrometry method. The intracellular levels of dFdCTP were determined by HPLC-UV in the WBC of treated pts. **Results**. As detailed in the following table, G pharmacokinetic was not affected by C on day 8.

				Day 1		Day 8	
Group	**course**	**Dose (mg/m^2)**		**C_{max} (μM)**	**AUC_{max} (μg*h/ml)**	**C_{max} (μM)**	**AUC_{max} (μg*h/ml)**
1	1	600	**Mean (SD)**	23.9 (± 2.5)	7.7 (± 1.0)	22.5 (± 4.7)	6.4 (± 1.5)
	2	1200	**Mean (SD)**	34.6 (± 20.0)	17.1 (± 5.8)	27.5 (± 5.0)	14.4 (± 2.3)
2	1	1200	**Mean (SD)**	18.7 (± 1.9)	10.5 (± 0.8)	21.2 (± 7.1)	10.4 (± 3.2)
	2	1200	**Mean (SD)**	18.0 (± 4.1)	9.2 (± 2.8)	21.6 (± 12.5)	11.1 (± 5.0)

Similar results were observed assessing intracellular Cmax of G triphosphate in peripheral blood mononuclear cells. Each infusion achieved the steady state concentration in all patients, with G half-life consistently lower than 30 minutes. Target plasma concentration of 15 μM was achieved in all courses, among patients treated with 600 mg/m^2 and in 14/16 courses, among those treated with 1200 mg/m^2 (range D1: 17.1-64.2 μM; D8: 15-39.8 μM). **Conclusions**. It appears from this study that both plasma and intracellular G concentrations over the time do not seem influenced by the C administration. Furthermore, G PI, at a FDR of 10 mg/m^2/min, may provide a therapeutic 15μM plasma G concentration to the large majority of patients.

P.02b

Antiproliferative effects of oxaliplatin and cisplatin can be enhanced using acylfulven analogues in human colon and breast cancer cell lines

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Background. Cisplatin (CIS) and oxaliplatine (OXA) are widely used for the treatment of many cancer types both as single agents and in combination with other anticancer drugs. Irofulven (IF; 6-hydroxymethylacylfulvene, MGI114) is a novel DNA-interacting anticancer agent that entered phase II/III clinical trials. To increase IF stability and reduce toxicity, a new analog N-hydroxy-N-(methylacylfulvene) urea (MGI4184) was synthetized. In this study we examined the cytotoxic effects obtained by combining OXA and CIS with IF and MGI4184.

Material and Methods. Antiproliferative effects of drugs were evaluated in a panel of human colon, breast and ovarian cancer cell lines using MTT assay. Combination studies were performed using the median effect plot analysis (Chou & Talalay).

Results. Exposure of cancer cells to OXA and CIS for 24 hours led to concentration- and time-dependent antiproliferative effects, CAOV3, HT29, and HT29/IF2 cancer cell lines being the most sensitive (IC50s 4-5µM) to oxaliplatin, while BT474, ZR751, and SKBR3 displayed more marked resistance (IC50s 50-150µM). Single agent IF given for 1 hour displayed cytotoxic effects against human cancer cell lines with IC50s ranging 1.3–92 µg/ml, HT29 being the most sensitive cell line. MGI4184 displayed similar activity than IF but appeared more stable with 18% of retained activity (versus 0.5% for IF) after 24 hours incubation. In HT29 colon cancer cells, IF and MGI4184 induce cell cycle arrest in G1/S phase and apoptosis. In HT29, the combination of OXA with IF (Table) or with MGI4184 led to synergistic activity. Experiments conducted in HT29/IF2 cells that were selected for acquired resistance to IF (80 fold-resistance as compared with parental HT29), showed only slight decrease in the synergistic activity of IF-OXA combination suggesting that mechanisms involved in resistance to IF do not impaire sensitivity to IF-platinum combinations.

Cell line	IF/OXA combinations		
	IF then OXA	OXA then IF	OXA+IF
HT29	Synergy	Synergy	Additive/Synergy
HT29/IF2	Additive/Synergy	Additive/Synergy	Additive
MCF7	Synergy	Additive/Synergy	Synergy
CAOV3	Additive	Additive/Synergy	Additive

Combinations of IF with CIS had similar effects than that of IF with OXA. Cell cycle analysis showed that CIS-IF and OXA-IF combinations may block cells in G1/S and potently induce apoptosis. Conclusions. OXA and CIS display synergistic anti-proliferative effects when combined with acylfulvene analogues over a broad range of concentrations in human colon and breast cancer cells. Acquired resistance to IF has limited impact on the effects of the combination

P.03b

Does cisplatin react primarily with cellular glutathione? A study of the interactions of cisplatin with cancer cell extracts

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The anticancer drug cisplatin (and its close congener carboplatin) is one of the three most extensively used anticancer drugs. Cisplatin and carboplatin are administered intravenously, but only a minority of the molecules finds its way to the cancer cells, penetrates them and reacts with one or more cellular components. Only 1% of the cellular Pt ends up on the DNA, its critical pharmacological target.
Much of the understanding about the mechanism of action of the drugs is derived from data gathered from aqueous model systems simulating "physiological conditions". These systems cannot reflect differences in various cancer cell lines. We have chosen to use extracts of cancer cells as models for the cytosols of the cancer cells. In order to better understand the cellular mode of action of platinum complexes we tried to follow the interactions of cisplatin with the cellular extracts.
For that purpose we prepared cis-diamminedichloropatinum(II) labeled with 15N and cis-dimethylaminedichloropatinum(II) labeled with 13C for 2D-HSQC NMR studies.1 Using 2D NMR we observed and compared the interactions between the drugs and extracts of cancer cells sensitive and resistant to cisplatin. Based on the literature, we expected to observe both aquated Pt(II) species and sulfur bound adducts (Ishikawa reported that 65% of the cellular Pt becomes bound to glutathione2). Interestingly we did not observe any aquated species of cisplatin nor interactions with sulfur nucleophiles. To gain better insights we studied the interactions of the drugs with different fractions of the cytosol (low molecular weight, cationic and anionic).

References

1. Gibson, D.; Kasherman, Y.; Kowarski, D.; Freikman, I., J Biol Inorg Chem 2006, 11 (2): 179-88.
2. Ishikawa, T.; Ali-Osman, F., J Biol Chem 1993, 268 (27): 20116-25.

P.04b

Modulation of survival pathways in ovarian carcinoma cell lines resistant to platinum compounds

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Since cytotoxic stress elicits various signalling pathways which may be implicated in cell survival or cell death, their alterations may have relevance in drug resistance. Thus, in the present study we investigated cell response to the EGF-R inhibitor gefitinib of ovarian carcinoma cell lines, including cells selected for resistance to cisplatin (IGROV-1/Pt1) and oxaliplatin (IGROV-1/OHP). Resistant sublines exhibited a marked decrease in sensitivity to gefitinib and resistance to apoptosis. Gefitinib was capable of inhibiting the phosphorylation of EGF-R in all the studied cell lines. The Akt and ERK1/2 kinases which act downstream of EGF-R were constitutively active in the three cell lines, but phospho-ERK1/2 levels were increased in the two resistant variants. This feature was associated with reduced sensitivity to the MEK1/2 inhibitor U0126. Gefitinib was more effective in inhibiting ERK1/2 and Akt phosphorylation in IGROV-1 cells than in IGROV-1/Pt1 and IGROV-1/OHP cells. Phospho-p38 was up-regulated in the resistant sublines, indicating the concomitant activation of distinct mitogen activated protein kinases. The up-regulation of phospho-p38 was associated with a peculiar localization of EGF-R which, in resistant sublines, was mainly internalized. In conclusion, our results indicate that the development of resistance to platinum drugs is associated with multiple alterations including deregulation of survival pathways activated by EGF-R resulting in a reduced cellular response to gefitinib.

P.05b

The effect of equatorial ligands on the reduction of platinum(IV) complexes by cancer cell extracts

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Pt(IV) complexes are inert octahedral compounds that are often used as prodrugs for the more cytotoxic Pt(II) analogues. The reduction potential of the Pt(IV) complex strongly depends on the nature of the axial ligands. It was suggested that the rate of reduction of Pt(IV) complexes depends also on the bulkiness of the equatorial ligands.[1]
Interestingly, two of the most active compounds have one bulky equatorial amine ligand that is *cis* to an NH_3 ligand. (see fig. 1).

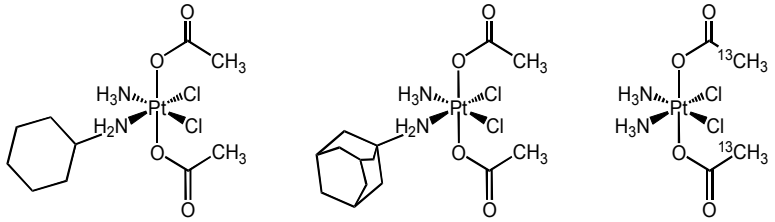

Figure 1: Pt(IV) complexes with axial acetate ligands. Satraplatin (left) and the complex with the adamantylamine ligand (center) are very active.

We have recently shown that the rate of reduction of ctc-[PtCl2(CH3CO2)(NH3)2] by cancer cell extracts depends on the type of cancer cell. Thus, studies that were conducted in aqueous solutions having "physiological conditions" (37°C, pH=7.4), neglected to consider the effect of the unique contents of each cancer cell line on the reduction of Pt(IV) complexes.
Our goal was to study the influence of the amine ligands on the rate of reduction of Pt(IV) complexes with axial acetate ligands in extracts of different types of cancer cells. Towards this end we prepared a Pt(IV) complex ctc-[PtCl2(13CH3CO2)2(NH3)(Am)] where Am=cyclohexyl amine, pyridine, isopropyl amine and n-butylamine labeled with 13C so that the reduction can be monitored by 2D-HSQC NMR.

We will report on the reduction rates of these compounds by aqueous extracts of three types of cancer cell lines: A2780 (ovarian cancer cells sensitive to cisplatin), A2780cisR (the cisplatin resistant line) and HT-29 (colon cancer cells).

References

1 Choi, S.; Filotto, C.; Bisanzo, M.; Delaney, S.; Lagasee, D.; Whitworth, J. L.; Jusko, A.; Li, C. R.; Wood, N. A.; Willingham, J.; Schwenker, A.; Spaulding, K. Inorg Chem 1998, 37, 2500-2504

P.06b

Differential profile of response to oxaliplatin/cetuximab combination highlights the initiation of DNA replication as a critical step of synergistic interaction

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We previously published that cetuximab enhances oxaliplatin antitumor activity in a panel of colorectal cancer cell lines regardless of the EGFR expression status but correlated with the level of phospho-EGFR in serum starved condition (Balin-Gauthier D. et al., Cancer Chemother Pharmacol. 2006). We demonstrated that oxaliplatin plus cetuximab combination was synergistic in HCT-8 cell line in vitro and in vivo while the HCT-116 cell line was unresponsive. But the mechanisms responsible for the combined effects of cetuximab with oxaliplatin remained unclear.

The aim of this study was to elucidate the interactions between the EGFR pathway and oxaliplatin pharmacology in order to determine how cetuximab enhances oxaliplatin effect in HCT-8 but not in HCT-116 colorectal cancer cell lines.

When a synergistic interaction occurred between oxaliplatin and cetuximab in the responsive HCT-8 cells, platinum–DNA adduct formation increased by 20%, suggesting two different, but possibly linked mechanisms 1) down regulation of the oxaliplatin detoxification pathway (GST) and/or 2) cetuximab inhibits the DNA-platinum adduct repair pathway. We have first studied the modulation of the GST activity, without displaying modification. We focused then on oxaliplatin–DNA adduct repair whose main cellular process is the nucleotide excision repair (NER) and its key enzyme ERCC1. We observed that inhibition of the NER remains a major pathway in the synergistic effect of the combination of the cetuximab to oxaliplatin in the sensitive HCT-8 cancer cells through the ERCC1 inhibition at the translational and transcriptional level. Deficiency of DNA repair drives cells to undergo apoptosis. We observed in HCT-8 cells exposed to oxaliplatin/cetuximab combination, morphologic changes associated with apoptosis (DNA condensation and fragmentation) concomitant with the inhibition of the oxaliplatin-induced survival pathway activation (AKT).

We also observed a reduced expression of factors involved in DNA replication initiation, which correlates with an enrichment of cells in the G1 phase of the cell cycle as well as an acceleration of apoptosis in HCT-8 cells. These new results suggest that the initiation of the DNA replication is a critical step of the synergistic response to cetuximab /oxaliplatin combination in the HCT-8 cell line.

P.07b

Reaction of cisplatin, carboplatin and oxaliplatin with the organic cation transporter antagonist cimetidine

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BACKGROUND*:* The organic cation membrane transporters (OCT) are expressed primarily in the liver (OCT1) and in the kidney (OCT2) in humans[1]. The interaction of platinum anticancer drugs e.g. cisplatin, carboplatin and oxaliplatin with these transporters has recently been evaluated with discordant results[2,3,4]. The specificity of the transporters has in many cases been studied using the competitive antagonist cimetidine under the assumption that it does not chemically interact with the platinum containing drugs.
METHODS*:* The degradation of the platinum drugs was studied in HEPES buffer pH 7.4 at 37 ºC using varying concentrations of cimetidine. The analysis of cisplatin, carboplatin and oxaliplatin was performed by liquid chromatography and post-column derivatization with N,N-diethyldithiocarbamate using microwave heating.
RESULTS*:* The second-order rate constants for the reaction of cimetidine with cisplatin, oxaliplatin and carboplatin were 1.28, 2.49 and 0.12 $M^{-1}\times min^{-1}$, respectively. For example, the degradation half-life of oxaliplatin is only 4.6 h in the presence of 1 mM cimetidine.
CONCLUSION*:* Caution should be taken when interpreting data from studies where the combination of platinum drugs and cimetidine are used taken into account the reactivity of cimetidine.

References
[1] Jonker JW and Schinkel AH. J Pharmacol Exp Ther 2004; 308: 2-9
[2] Ciarimboli et al. Am J Pathol 2005; 167: 1477-1484
[3] Yonezawa et al. J Pharmacol Exp Ther 2006; 319: 879-886
[4] Zhang S et al. Cancer Res 2006; 66: 8847-8857

P.08b

Interaction of photoactivated platinum compounds with bacteriophage

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Cisplatin is a well known and effective chemotherapeutic drug, which is highly toxic to cancer cells, by forming DNA lesions and inducing apoptosis [1]. However, cisplatin and related Pt(II) analogues have a number of accompanying side-effects. Therefore, Pt(IV) diazido diam(m)ino complexes of the form [Pt(N3)2(OH)2(X)2] where X = e.g. NH3, have been developed as relatively non-toxic prodrugs which can be light-activated at the tumour site, with the hope of minimising unwanted side-effects [2].

Cisplatin also exhibits antiviral activity against a number of bacterial viruses (also known as bacteriophage) [3]. We have been using the dsDNA bacteriophage P1 and its host Escherichia coli as a model system to investigate the antiviral activity of two photoactivatable Pt(IV) diazido diam(m)ino complexes, cis, trans, cis-[Pt(N3)2(OH)2(NH3)2] and trans, trans, trans-[Pt(N3)2(OH)2(NH3)2]. P1 is normally able to lyse E. coli cells. However, we determined that upon light activation, these complexes reduced the ability of P1 to subsequently lyse E. coli cells in a time and concentration dependent manner. In contrast, neither light activation alone nor dark-treated complexes significantly affected the lytic ability of P1. Preliminary SDS-PAGE studies of purified P1 particles, suggested light activation alone affects the P1 proteins, despite not affecting the lytic ability of P1. However, since the complexes appeared to protect the P1 proteins from the effects of light activation, this data raises the possibility that photoactivation can induce platination of specific proteins in the bacteriophage coat thereby shielding the proteins from the effects of light activation alone. We are investigating this hypothesis and examining the effect of the complexes on the dsDNA of P1.

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[1] J. Reedijk, Chem. Commun. 1996, 801-806.
[2] P.J. Bednarski, F.S. Mackay and P.J. Sadler, Anti-Cancer Agents Med. Chem. 2007, 7, 75-93.
[3] J. Drobník, A. Blahušková, S. Vasiluková and A. Krekulová, Chem.-Biol. Interactions, 1975, 11, 365-375.

P.09b

Anticancer osmium(II) arene complexes containing picolinic acid derivatives as chelating ligands

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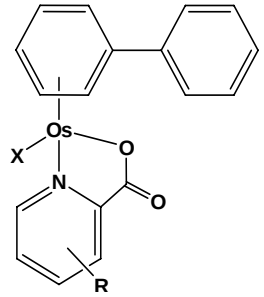
Recent work on osmium(II) compounds of the type [(arene)Os(YZ)X] has shown that their aqueous reactivity is highly dependent on the nature of the chelating ligand YZ and the leaving group X.1 When YZ is N,N the resulting complex hydrolyses about 40 times slower compared to the ruthenium analogues, but when YZ is N,O or O,O the hydrolysis occurs at a rate similar to that of the ruthenium analogues. Moreover, several complexes containing N,O chelating ligands show promising cytotoxic activity in the human ovarian cancer cell line.2 This study attempts to 'fine-tune' the reactivity of the osmium metal centre using picolinate chelating ligand derivatives with different substituents R (Figure) on the ortho and para position which vary in steric bulk and electronic properties. Solid state structures, cytotoxicity data and investigations of their aqueous chemistry using 1H NMR spectroscopy, including their rates of hydrolysis, acidity of the resulting aqua adducts and nucleobase binding studies are discussed. It is found that the different ortho and para substituents strongly effect the hydrolytic behaviour of the resulting complexes, especially with respect to hydrolysis rates and binding to model nucleobases. In addition, two compounds show promising cytotoxic activity.



Figure

P.10b

Effect of Pd(II) and Pt(II) complexes with condensation products of 2-(diphenylphosphino)benzaldehyde on activation of caspase-3 and migratory potential of HELA cells

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In our previous in vitro studies on cancer cells we identified novel metal complexes: palladium(II) complex of the condensation product of 2-(diphenylphosphino)benzaldehyde (dpba) and ethyl hidrazinoacetate (etha) and two related Pd(II) and Pt(II) complexes of the condensation product of 2-(diphenylphosphino)benzaldehyde (dpba) and semioxamazide as cytotoxic and potential MMP-2 inhibiting agents, with activity comparable to cisplatin (J. Inorg. Biochem. 100: 1811-1818, 2006.) In order to analyze mechanism of their antimetastatic activity we performed cell migration assay using double compartment tissue culture plates (BD, Bioscience, USA) with polycarbonate membrane inserts (8 μm pore size). HeLa cells were treated for 24 h continuously with investigated complexes in two concentrations IC50 and 50 μM. Migrated cells are detached from the bottom of the polycarbonate membrane and labeled with fluorescent dye. We found that investigated complexes significantly reduce migration of Hela cells (up to 33%) in a dose-dependent manner compared to control (untreated cells).

We also investigated influence of these complexes on expression level of apoptosis related protein caspase-3 at HeLa cells by Western blot. After 24 h of continuous action of investigated complexes (in concentrations of 0.5 x IC50 and IC50) slight expression of activated caspase-3 was detected.

This data suggest that novel palladium(II) and platinum(II) complexes exhibited apoptotic and anti-migratory potential in vitro, thus giving potential for further investigation.

P.11b

Potential anticancer platinum –pyrimidine complexes: DNA-interaction studies

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Metallodrugs containing "platinum" have enormous potential for clinical applications. Cisplatin, carboplatin and oxaliplatin are being used successfully in cancer chemotherapy and they share a common structural feature of having Pt(II) and square-planar geometry rendered by the ligands. The targets of the platinum-anticancer drugs in vivo are genomic DNA, cellular proteins, or S-containing scavengers (like glutathione and metallothionein). Some platinum-pyrimidine complexes are already known in literature as promising antitumor complexes. In addition some platinum-intercalators display increased antitumor activity and reduced side-effects when compared to cisplatin.1
These results inspired to the synthesis of the platinum complexes of a new bis-pyrimidine ligand.2 The fine-tuning of carrier ligands led to a series of interesting complexes. The changes in the structure of the carrier ligands can influence the biological activity and DNA binding affinity.3 Some of these complexes are studied to investigate the mode of interaction towards DNA by CD and UV-VIS spectroscopy. To investigate the interaction with proteins (albumin and transferrin) ESI-MS is used.4 The reactions with model bases are followed by ESI-MS and NMR spectroscopy. The results from the DNA and protein interaction, as well as cytotoxicites towards certain cancer cell-lines, will be presented.

References

1. Wheate, N. J.; Brodie, C. R.; Collins, J. G.; Kemp, S.; Aldrich-Wright, J. R. Mini-Rev. Med. Chem. 2007, 7, 627-648.
2. Yao, W. B.; Kavallieratos, K.; de Gala, S.; Crabtree, R. H. Inorg. Chim. Acta 2000, 311, 45-49.
3. Fisher, D. M.; Bednarski, P. J.; Grunert, R.; Turner, P.; Fenton, R. R.; Aldrich-Wright, J. R. ChemMedChem 2007, 2, 488-495.
4. Casini, A.; Gabbiani, C.; Mastrobuoni, G.; Messori, L.; Moneti, G.; Pieraccini, G. ChemMedChem 2006, 1, 413-417.

P.12b

Pharmacokinetic studies of intraperitoneal oxaliplatin using liquid chromatography with post-column derivatization

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BACKGROUND: A new therapeutic approach has lead to a long-term survival of some cases of peritoneal carcinomatosis (PC). The treatment comprises cytoreductive surgery of PC followed by hyperthermic intraperitoneal chemotherapy (HIPEC) with oxaliplatin1. Pharmacokinetic studies have so far only been based on the use of an unselective analytical technique e.g. atomic absorption spectroscopy (AAS) measuring the total platinum content.
METHODS: HIPEC was carried out in 8 patients by the open abdomen Coliseum technique for 30 min at 41.5 - 43 ºC with 460 mg/m2 of oxaliplatin in 5% dextrose solution. Blood and perfusate samples were collected during the perfusion. Additional blood samples were taken up to 2 h after the end of the perfusion. All samples were immediately placed on ice and ultrafiltrated centripetally at 4 ºC. Before analysis the perfusion samples were diluted 100-fold. The analysis was performed by liquid chromatography and post-column derivatization with N,N-diethyldithiocarbamate using microwave heating2.
RESULTS: The mean elimination half-life of oxaliplatin in the perfusate was 29.5 min (range 21.1 - 41.2 min) and 24.7 min (range 21.7 - 27.7 min) in the peripheral circulation. The area under the time concentration curve (AUC) in blood was 9.2 ± 2.0 % (SD) of that in the perfusate. The dose corrected AUC in blood was 2.57 ± 0.35 (SD) µM×min/mg/m2 as compared to previous results 9.90 µM×min/mg/m2 obtained by AAS1.
CONCLUSION: The use of AAS grossly overestimates the systemic exposure of oxaliplatin after HIPEC as compared to the results obtained by a selective analytical technique.

References

1. Elias D et al. Ann Oncol 2004; 15: 1558-1565; 2Ehrsson H, Wallin I. J Chromatogr B 2003; 795: 291-294

P.13b

Salvage chemotherapy containing nedaplatin, 2nd generation derivative of CDDP, for CDDP resistant or refractory germ cell tumors

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INTRODUCTION: Only 20 - 30 % of patients with cisplatin (CDDP) refractory germ cell tumor (GCT) will remain continuously disease free with salvage chemotherapy. The present study investigated the combination chemotherapy with nedaplatin (CDGP), second generation derivative of CDDP developed in Japan, as salvage chemotherapy for CDDP refractory GCT.
METHODS: Between June 1998 and August 2006, 58 patients with CDDP refractory GCT were assessed, retrospectively. These patients were treated by CDGP N in combination with Paclitaxel (T), Ifosphamide (I), CPT-11 (Ir) and/or Gemcitabine (G). TIN therapy consisted of T: 210 mg/m2 on day 1, N: 100 mg/m2 on day 2 and I: 1.2g/m2 on day 2 to 6 every three weeks. IrN therapy consisted of Ir: 100-150mg/m2 on day 1, 15 or 200-300 mg/m2 on day 2, N: 100 mg/m2 on day 1 every four weeks. TGN therapy consisted of T: 210 mg/m2 on day 1, N: 100 mg/m2 on day 2 and G: 1000mg/m2 on day 1 and 8 every three weeks. TIN, IrN and TGN combination therapies were performed as the second-line or more chemotherapy in 34, 30 and 14 cases, respectively.
RESULTS: The response rate of TIN, IrN and TGN therapy was 68.5% (CR: 0%, PRm-: 50.9%, PRm+ : 17.6%), 56.7% (CR: 6.7%, PRm-: 40%, PRm+: 10%) and 57.1% (CR : 0%, PRm-: 21.4%, PRm+: 35.7%), respectively. The outcome of these patients were NED 48.2, AWD 22.4 and Dead 29.4%, respectively. Major adverse events more than grade 3 were neutropenia, thrombocytopenia. Grade 4 peripheral neuropathy was observed in one patient. No renal toxicity was observed in these combination therapies. Grade 4 diarrhea which was major adverse event of CPT-11 was observed in only one case.
CONCLUSION: This study demonstrates that combination chemotherapy with CDGP showed significant anticancer activity for patients with CDDP refractory GCT without severe adverse events. These findings suggest that salvage chemotherapy containing CDGP may be one of the options of salvage chemotherapy for CDDP refractory GCT.

Abbreviation: CR, complete response PRm- , partial response with marker normalization PRm+, partial response without marker normalization

P.14b

Voltage-gated potassium channels and oxaliplatin neurotoxicity

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BACKGROUND: The dose-limiting toxicity of oxaliplatin therapy is neurotoxicity. Acute neurotoxicity manifests as tingling and creeping sensations of the distal extremities and appears during or shortly after infusion. These symptoms might be related to hyperexcitability of sensory neurons and there are studies on effects of oxaliplatin on voltage-gated ion channels. One group observed effects on voltage-gated potassium channels1. It has been suggested that biotransformation products of oxaliplatin could be responsible for the neurotoxic side effects. For example, complexes with chloride have been identified in blood from patients undergoing oxaliplatin therapy. We hypothesized that a ring-opened negatively charged chloride2 complex can affect voltage-gated ion channels. The reaction of oxaliplatin with endogenous sulfur-containing compounds is rapid3. We also examined the effect of oxaliplatin on potassium channel subtypes with target cysteines on the channel protein4.
METHODS: The experiments were performed on Shaker H4 channels5. cRNA was transcribed and injected in Xenopus laevis oocytes as prevoiusly described4. The currents were measured with a two-electrode voltage-clamp technique. A mix of oxaliplatin, the dichloro complex, and the monochloro monooxalato complex of oxaliplatin was produced by adding sodium chloride to an oxaliplatin solution2.
RESULTS: There were no detectable effects of extracellularly applied oxaliplatin (up to 120 μM) on either the time courses or the amplitudes of potassium currents. The channel´s voltage dependence was not affected (<0.5 mV). Experiments were carried out in both chloride-containing and chloride-free extracellular solutions. Addition of the monochloro monooxalato complex-mix did not affect the result. Neither did we detect any effects on the potassium channel mutations with cysteines at critical positions.
CONCLUSION: Our result does not support previous findings of oxaliplatin effects on voltage-gated potassium channels.

References

1. Benoit E et al. Gen Physiol Biophys. 2006; 25: 263-76.
2. Jerremalm E et al. Pharm Res. 2004; 21: 891-4.
3. Jerremalm E et al. Eur J Pharm Sci. 2006; 28: 278-83.
4. Larsson HP and Elinder F. Neuron. 2000; 27: 257-83.
5. Kamb A et al. Cell. 1987; 50: 405-13.

P.15b

Neurotoxicity of platinum compounds: comparison of the effects of cisplatin and [PT(O,O'-ACAC)(g-ACAC)(DMS)]

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The widely used cisplatin has significant side effects and acquired or intrinsic drug resistance, limiting its clinical application; thus, much attention has been focused on designing new Pt compounds with improved pharmacological properties. In this context, a novel Pt(II) complex, [Pt(*O,O'*-acac)(γ-acac)(DMS)] (acac), was synthesized [1], whose activity may not necessarily require reaction with DNA [2]. In this study we examined the ability of acac and cisplatin to induce cell death in human neuroblastoma cell line SH-SY5Y, and their neurotoxicity on the rat cerebellum during postnatal development. **Methods**. SH-SY5Y were treated with increasing doses (1-200μM) and for different incubation times (1-72h) with acac and cisplatin, and the number of survived cells evaluated by MTT. The cleavage of caspase-3, -7, and Poly-ADP ribose polymerase (PARP) were analysed by Western methods. Ten-day-old rats were injected according previous experimental plan (5μg/g b.w.); paraformaldehyde perfused cerebella were taken at 6h, 24h, and 7 days after treatment. PCNA-, GFAP-, Calbindin-immunoreactions, and TUNEL were performed. **Results**. Both acac and cisplatin provoked a dose-dependent decrease of viable cell number, but the IC_{50} values (drug concentrations required for 50% growth inhibition) at 24 and 48h of incubation for acac (10 and 7μM, respectively) were lower than those for cisplatin (75 and 50μM, respectively). About 25% of cells died after 1h treatment with 100μM acac whilst occurred 9h to get the same result using 100μM cisplatin. Both cisplatin and acac caused cell death by apoptosis inasmuch as the proteolysis of procaspase-3, -7 and PARP were detected in treated cells. In the cerebellar cortex, at 6h from the injection, the incidence of apoptotic cells in the germinative layer was similar after acac and cisplatin, as well as the alterations of radial glia fibres and Purkinje neuron dendrites. At 24h, a lower number of apoptotic cells were found after acac in the germinative layer, where the premigratory zone was similar to control rats. At 7 days, the dendrites of Purkinje neurons appeared more ramified after acac. **Conclusion**. Acac has a cytotoxic activity *in vitro* much higher than cisplatin, with apoptosis being the cell death caused in SH-SY5Y cells. The data on the developing CNS suggest that acac could have less neurotoxic effects than cisplatin, since induces less apoptosis among proliferating neural cells, also with higher doses (e.g. 10μg/g b.w.), less altered neuronal migration and dendrite growth; future studies including more restricted times from the injection will be needed to support this hypothesis.

References

[1] De Pascali SA et al., Eur J Inorg Chem 2005; 788–796.

[2] Muscella A et al., Biochem Pharmacol 2007; 74:28-40

P.16b

Excessive formation of platinum-DNA adducts in distinct target cells of cisplatin toxicity: role of active uptake systems

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The efficacy of cancer chemotherapy with platinum complexes is hampered by severe and dose-limiting side effects. The pathophysiological mechanisms leading to damage and functional loss in terminally differentiated cells of normal tissue and the cell type specificity, however, are still poorly understood. Therefore, we have set up a highly sensitive antibody-based technique to measure formation and repair rates of structurally defined Pt-adducts in the nuclear DNA of individual cells. As patients frequently suffer from pronounced ototoxicity and permanent hearing loss after treatment with cisplatin we have applied this method in a mouse model to quantify the level of DNA adducts in cells of the inner ear. Although all cells of that anatomical structure exhibited comparable capabilities to repair cisplatin-induced DNA intrastrand cross links two distinct cell types depicted extraordinary high accumulation of those lesions, the marginal cells of the stria vascularis and the outer hair cells of the organ of Corti. Carboplatin which induces exactly the same set of adducts neither shows severe ototoxicity nor a similar pronounced adduct formation in distinct cochlear cells. From this observation we conclude that excessive DNA platination in the marginal and outer hair cells represents the earliest event in short-term cisplatin ototoxicity, which then triggers their functional impairment and/or their potential apoptotic destruction. As both target cells are characterized by their very high activity for ion transport we suggest an efficient uptake of cisplatin by distinct membrane transporters into those cells as the major reason for their vulnerability by the drug. By cell type-specific expression analysis and by application of potential pharmacological inhibition we now try to further characterize the responsible membrane transporters to pave the way for efficient oto-protective strategies during antineoplastic treatment schedules with platinum drugs.

P.17b

Long-term platinum exposure after treatment with oxaliplatin and cisplatin

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Background: The purpose of this study was to evaluate long-term platinum exposure in patients previously treated with oxaliplatin and cisplatin. **Methods**: Forty-five patients, treated with oxaliplatin or cisplatin 8–75 months before participating in this study, were included. Platinum (Pt) levels in plasma, plasma ultrafiltrate (pUF), and bound to DNA in peripheral blood mononuclear cells (PBMCs) were assessed using inductively coupled plasma mass spectrometry. Possible factors affecting Pt retention were collected, including cumulative dose, follow-up time, serum creatinine (SC), glutathione S-transferase (GST) genotypes, and co-administration of sodium thiosulfate (STS with intra-arterial (i.a.) cisplatin). Results were evaluated using non linear mixed effects modelling. **Results**: Twenty-five colorectal cancer patients were treated with cumulative oxaliplatin doses of 585–1520 mg/m2 (corresponding to 287–747 mg/m2 Pt). Twenty patients were treated with cisplatin for various cancer types (9 testis, 8 head-neck, 1 NSCLC, 1 SCLC, and 1 yolk sac) with cumulative doses of 300–600 mg/m2 (corresponding to 195–390 mg/m2 Pt). Five cisplatin patients received STS. Pt plasma concentrations ranged between 460–2993 ng/l and 142–1154 ng/l for oxaliplatin and cisplatin respectively. For oxaliplatin, on average 24.2% of the plasma Pt was recovered in ultrafiltrate, whereas for cisplatin this recovery was 14.6%. No DNA bound Pt in PBMCs could be detected in any of the patients. Ex vivo incubation of DNAwith pUF of patients and with blank pUF spiked with equivalent concentrations of cisplatin and oxaliplatin showed that less than 10% (equal to limit of quantification of the assay) reactivity of Pt species in patients pUF was retained compared with the parent compounds. Pt levels showed a decrease with time. The observed overlap of follow-up time for oxaliplatin and cisplatin (8–23 vs 18–75 months) justified combination of the data. The decrease of Pt levels was characterized by two elimination times (t1/2a = 3.54 months and t1/2(1 = 21.5 months). Pt levels were related to cumulative dose. Moreover, Pt levels were reduced by 30.4% with i.a. cisplatin and co-administration of STS ($P < 0.0001$). Median SC levels before start of the Pt chemotherapy were 81 and 76 lmol/l for oxaliplatin and cisplatin, respectively. At the time of the current study, SC levels of cisplatin patients were increased to 96 lmol/l, whereas oxaliplatin SC levels remained constant (87 lmol/l). Pt levels were significantly related to SC levels before start of Pt chemotherapy ($P = 0.01$). No relationship between GST genotypes and Pt levels was observed which could be due to the small sample size. **Conclusions**: Our data suggest that plasma and pUF Pt levels are related to follow-up time, cumulative dose, SC and STS use. The >90% reduction in reactivity of Pt in pUF suggests that Pt in pUF is mainly bound to various small proteins, protein fractions, and other small molecules.

P.18b

Bimonthly chemotherapy with oxaliplatin, irinotecan, infusional 5-fluorouracil/folinic acid in patients with metastatic colorectal cancer pretreated with Cpt-11- or OHP-based chemotherapy

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BACKGROUND*:* This study was conducted to assess the tolerability and efficacy of a ternary bimonthly irinotecan (CPT-11) - oxaliplatin (OHP) -infusional 5-fluorouracil (5-FU)/folinic acid (FA) combination in advanced colorectal cancer patients who had received prior CPT-11 and/or OHP-based chemotherapy regimen.

PATIENTS AND METHODS*:* Colorectal cancer patients were given bimonthly CPT-11 as a 90-min infusion, followed by OHP (85mg/m^2), FA (200mg/m^2) 2-h infusions and 5-FU (48-h infusion). CPT-11 and 5-FU dose were escalated as reported below.

RESULTS*:* 26 patients were recruited. Fourteen patients had received a prior CPT-11-, 6 patients a prior OHP-based chemotherapy regimen and 6 patients both regimens. Three dose levels were investigated: CPT-11 100, 120 and 140mg/m^2 and 5-FU 1500, 1800 and 2100mg/m^2 in 6, 12 and 8 patients, respectively. All patients were evaluable for toxicity, 24 for antitumour activity. All dose levels (188 cycles) were well tolerated with acceptable toxicity. Grade 4 toxicity occurred in two patients only (neutropenia in one case and stomatitis in onother one, 3.8%). Grade 3 toxicities included: nausea and vomiting (34.6%), asthenia (26.9%), neurosensory toxicity (15.4%), neutropenia (3.8%) and diarrhea (3.8%). Hematological toxicity was not frequent and generally mild. At the third dose level, a higher, although not significantly different incidence of hematological and neurosensory toxicity (both occurring in 62.5% of cases, all grades) was observed compared the other two, while nausea and vomiting was significantly less frequent (37.5% vs 100%). We observed 2 complete responses, 9 partial responses (OR 45.8%), 8 stable disease (33.3%), and 5 disease progression (20.8%). Median overall survival was 18 months and median time-to-progression 5 months.

CONCLUSION*:* This combination showed limited toxicity and promising antitumor activity in CPT-11 and/or OHP pretreated colorectal cancer patients. The second dose level using CPT-11 at 120 mg/m^2 and 5-FU at 1800 mg/m^2 is recommended for further phase II studies in this patient population.

P.19b

Pharmacokinetic studies of polynuclear platinum complexes with plasma and human serum albumin: implications for drug design and delivery

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Metabolic interactions of platinum anticancer compounds can dictate the pharmacokinetic properties and ultimately the drugs' performance in the clinical setting. Among these interactions include coordination to and ultimately deactivation with plasma proteins. The triplatinum anticancer agent, BBR3464, (Figure 1) currently undergoing phase II clinical trials, interacts strongly with plasma proteins, causing a prolonged elimination half-life, resulting in reduced tolerability in patients.1,2 Interactions of polynuclear platinum compounds (PPCs), given the general structure {(Pt)(L)(NH3)2}X{(Pt)(L)(NH3)2}n+(Figure 1), with mouse and human plasma, as well as with individual plasma proteins such as human serum albumin have been studied. Among these, polynuclear platinum compounds with promising levels of protein binding and enhanced cytotoxicity are also discussed. Human serum albumin, know for its ability to transport various types of drugs and ligands, is discussed as a possible drug delivery system for a class of PPCs (Figure 1) analogous in structure to BBR3464. These compounds, Ia and Ib, contain substitutionally "inert" –NH3 groups and therefore may "by-pass" the deactivation associated with Pt-protein bond formation. Electrostatic interactions, not coordinate binding, predominate in Ia and Ib protein interactions.3 This is especially important for Ib. This 8+ compound is taken up into cells in a significantly greater concentration than either BBR3464 or Ia, and as a result has demonstrated in vitro cytotoxicity equivalent to cisplatin.

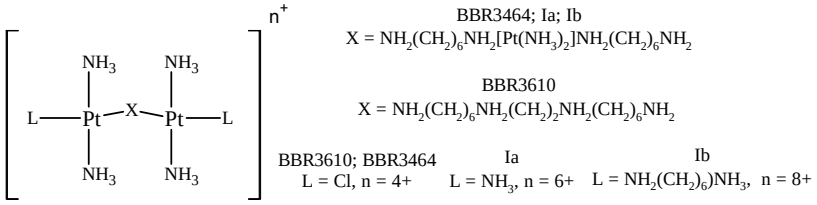

Figure 1: Structure of Polynuclear Platinum Compounds Studies

References

1. Sessa, C., Annals of Oncology 11: 977-983, 2000
2. Jodrell, D., European Journal of Cancer 40: 1872–1877, 2004
3. Montero, E., Dalton Trans., 2007, DOI: 10.1039/b708433c

P.20b

Docetaxel (D), cisplatin (C) and continuous infusion 5-fluoruracil (5-FU) for the treatment of locally advanced and metastatic gastric and esophageal cancer: reduced toxicity of a weekly schedule

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BACKGROUND Docetaxel, Cisplatin and 120-hour continuous infusion 5-FU administered every three weeks are considered standard treatment for advanced gastric cancer. However, the toxicity of the every-three weeks combination is a major issue. In this paper we report a retrospective evaluation of a simplified weekly schedule of D and C with prolonged infusion 5-FU.
METHODS Chemo-naive patients (pts) with gastric and esophageal cancer, either locally advanced or metastatic were treated according to the following schedule: T 35 mg/m2, C 25 mg/m2, and 7-day 5-FU prolonged venous infusion 180 mg/m^2/day, administered weekly over three consecutive weeks followed by 1 week rest. Locally advanced patients received a neoadjuvant chemo-radiation program. Radiotherapy was started on the first day of the second cycle of chemotherapy, at the daily dose of 1.8 Gy/f, 5 days/week for 5 consecutive weeks (total dose 45 Gy). During radiotherapy patients received the same chemotherapy scheme, without rest, with the dose of 5FU reduced to 150 mg/m^2/day. Surgery was performed 4-10 weeks from the end of treatment. The primary objective was to evaluate the safety profile and toxicity according to NCI common toxicity criteria.
RESULTS From April 2004 we included twenty pts: median age was 62 years (range 41-77) ; 11 pts had metastatic gastric cancer, 6 locally advanced gastric cancer, 2 had metastatic and 1 locally advanced esophageal cancer. Overall 52 cycles were administered to the 13 advanced pts (median number of cycles/patient = 4, range 2-6). Six pts treated according to the neoadjuvant program were able to receive full doses of chemo- and radiotherapy, one patient had a two-weeks delay because of G3 mucositis and G3 fatigue.
Reported (G3) adverse events were mucositis and anemia in 2 pts (10%), diarrhea in 1 pt (5%), neutropenia in 1 pt (5%) , alopecia in 1 pt (5%), fatigue in 1 pt (5%) and nail toxicity in 1 pt (5%). No grade 4 toxicity were observed. In 11/13 evaluable advanced pts we observed 7 Partial Responses (64%), 3 Stable Diseases (27%) and 1 Progressive Disease (9%) (RECIST criteria). Among the 6 locally advanced pts 4 could be radically resected. One pt was not resected because of progressive disease, and one because of clinical deterioration. One patients was not evaluated (too early).
CONCLUSION With the limitation of the small sample size, we conclude that weekly administration of D, C, and continuous infusion 5FU is active and presents a favorable toxicity profile. Combination with radiotherapy is safe with a minimal dose reduction of 5FU.

P.21b

Treatment of peritoneal carcinomatosis by cytoreductive surgery and intraperitoneal hyperthermic chemoperfusion (IHCP): postoperative outcome and risk factors for morbidity

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INTRODUCTION: The curative surgical treatment for peritoneal carcinomatosis is possibile through cytoreductive surgery, with limited or extended peritonectomy, associated with Hypertehermic IntraPeritoneal Chemotherapy (HIPEC).
OBJECTIVE: The purpose of this study was to analyze the procedure related to postoperative outcome in the cases of peritoneal carcinomatosis and/or positive cytology for neoplastic cells at peritoneal washing.
PATIENTS AND METHODS: Twenty-four patients with peritoneal carcinomatosis or positive cytology at peritoneal washing were treated in our Department from July 2005 to October 2007. One patient underwent the procedure twice because of early relapse of pseudomixoma peritonei. Primary tumor was ovarian carcinoma in ten patients: four cases presented peritoneal surface malignancies (PSM) after hysteroadnexectomy, six cases synchronous PSM.
Primary tumor was gastric cancer in seven patients: in four cases the neoplasm was T3/T4 with positive cytology at peritoneal washing and two cases presented PSM after gastrectomy. One patient was previously treated with ovariectomy for ovaric mass that resulted a Krukemberg's tumor of gastric cancer.
In four cases HIPEC was performed synchronously with primary tumor resection, in three cases after gastrectomy.
Primary tumor was pseudomixoma peritonei in four patients; cytoreductive surgery and HIPEC was carried as first line terapy in only one patient.
Three patients were previously treated for colon carcinoma.
The mean Peritoneal Cancer Index (PCI) was 14.
HIPEC was carried out through the abdominopelvic cavity for 60 minutes using a closed abdomen technique. The drugs used were Mitomycin C (3.3 mg/m2/L) and Cisplatin (25 mg/m2/L). The intracavitary mean temperature was 41.8°C.
RESULTS: Postoperative complications occurred in 10 (42%) cases: pancreatitis (4 cases), renal toxicity (3), cerebral hemorrhage (one case), evisceration (one case) and duodenal stump leakage after total gastrectomy (one case). In the last two cases reoperation was needed. Mean hospital stay was 11,5 days (6-35 days). No patients died in the postoperative period. With a mean follow-up of one year, 14 (58%) patients are alive and mean survival is 12,6 months (1-40 months)
CONCLUSIONS: Cytoreductive surgery associated with HIPEC is a good therapeutic option especially in ovaric and colic-related carcinosis, but the procedure has still a high post-operative morbidity, even though we had no mortality, and should be reserved to selected cases.

P.22b

Interaction of photoactivated platinum compounds with bacteriophage

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Cisplatin is a well known and effective chemotherapeutic drug, which is highly toxic to cancer cells, by forming DNA lesions and inducing apoptosis [1]. However, cisplatin and related Pt(II) analogues have a number of accompanying side-effects. Therefore, Pt(IV) diazido diam(m)ino complexes of the form $[Pt(N_3)_2(OH)_2(X)_2]$ where X = e.g. NH_3, have been developed as relatively non-toxic prodrugs which can be light-activated at the tumour site, with the hope of minimising unwanted side-effects [2].
Cisplatin also exhibits antiviral activity against a number of bacterial viruses (also known as bacteriophage) [3]. We have been using the dsDNA bacteriophage P1 and its host *Escherichia coli* as a model system to investigate the antiviral activity of two photoactivatable Pt(IV) diazido diam(m)ino complexes, *cis, trans, cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$. P1 is normally able to lyse *E. coli* cells. However, we determined that upon light activation, these complexes reduced the ability of P1 to subsequently lyse *E. coli* cells in a time and concentration dependent manner. In contrast, neither light activation alone nor dark-treated complexes significantly affected the lytic ability of P1. Preliminary SDS-PAGE studies of purified P1 particles, suggested light activation alone affects the P1 proteins, despite not affecting the lytic ability of P1. However, since the complexes appeared to protect the P1 proteins from the effects of light activation, this data raises the possibility that photoactivation can induce platination of specific proteins in the bacteriophage coat thereby shielding the proteins from the effects of light activation alone. We are investigating this hypothesis and examining the effect of the complexes on the dsDNA of P1.

We thank EPSRC, Edinburgh University and Warwick University (studentship - HP), BBRSC (studentship - VM) and Scottish Enterprise for support, and COST D39 for discussions.

References

[1] J. Reedijk, *Chem. Commun.* 1996, 801-806.
[2] P.J. Bednarski, F.S. Mackay and P.J. Sadler, *Anti-Cancer Agents Med. Chem.* 2007, **7**, 75-93.
[3] J. Drobník, A. Blahušková, S. Vasiluková and A. Krekulová, *Chem.-Biol. Interactions*, 1975, **11**, 365-375.

P.23b

Calcium-modulated release of active platinum antitumor compounds from sol-gel silica xerogels

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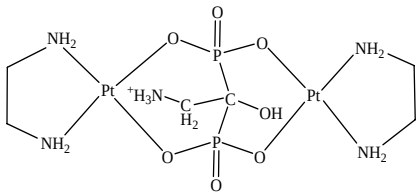
Second generation (Carboplatin) and third generation (Oxaliplatin) platinum complexes have been synthesized in the past 30 years to overcome Cisplatin toxicity and resistance or to improve its efficacy.[1] A promising approach to the development of new platinum-based cytostatic agents involves the use of carrier ligands able to promote the accumulation of the drug in target organs or cells. Several proofs of the concept have already been reported including a class of platinum complexes containing phosphonic acids.[2] Bisphosphonates show extremely high affinity for bones and other calcified tissues and are clinically used as therapeutic agents against Paget's disease, cancer-related hypercalcaemia, and post-menopausal osteoporosis. The combination of the bisphosphonic function with the platinum complex links together a cytostatic and a bone-targeting moiety.
A significant improvement of the potency of a platinum drug could also be achieved by a local and sustained administration at the diseased site (bone tumor or metastasis). To this end, the platinum compounds could be embedded into biocompatible and slowly resorbable polymeric matrices to be implanted *in situ* (e.g. by surgery).[3,4] In a previous work we have investigated the adsorption and desorption of cisplatin, alendronate, and a platinum complex containing the bisphosphonate medronate towards two bio-mimetic synthetic hydroxyapatite nanocrystal materials having different crystal shape and chemico-physical properties.[5] In the present study we report the synthesis of a platinum(II) complex with the ligand (2-amino-1-hydroxyethane-1,1-diyl)bis(phosponic acid).[6] The novel dinuclear Pt complex has been embedded into two xerogel matrices: the first composed of pure silica and the second containing also calcium, which could act as a release-modulating agent thanks to its affinity for the bisphosphonic moiety of the Pt complex. The matrix loading capability, the release of the Pt-complex in a physiological simulating buffer, and the stability upon storage were investigated as a function of the presence of calcium in the formulation and of the Pt-complex concentration.



References

[1] E. Wong and C.M. Giandomenico, *Chem. Rev*. **1999**, *99*, 2451. [2] M. Galanski, et al. *J. Med. Chem.* **2003**, *46*, 4946. [3] R. Baker, *Controlled Release of Biologically Active Agents*, **1987**, J.Wiley & Sons. [4] M. Morpurgo, et al. *Farmaco*, **2005**, *60*, 675. [5] B. Palazzo, M. Iafisco, M. Laforgia, N. Margiotta, G. Natile, C. L. Bianchi, D. Walsh, S. Mann, and N. Roveri. *Adv. Funct. Mat.* **2007**, *17*, 2180. [6] N. Margiotta, R. Ostuni, D. Teoli, M. Morpurgo, N. Realdon, B. Palazzo, G. Natile. *Dalton Trans.* **2007**, 3131.

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P.24b

ProLindac, a pH-dependent polymer-vectorized DACH, is safe at pharmacodynamically active doses: results from an ongoing Phase II trial in borderline potentially platinum-sensitive ovarian cancer (OC) patients

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Background: The Recommended Dose (RD) for ProLindac™ is 640 mg/m²/day1-8-15 q4w; Dose Intensity (DI): 480 mg/m²/week (Campone et al, 2007). Oxaliplatin has single-agent activity in OC. We aim to optimize dose and schedule (q3w and q2w) in this benchmark indication using a DI driven escalation scheme.

Patients and Methods: Patients (pts) having failed 2-4 prior chemotherapy (CT) lines were eligible provided they had ≥6 months of platinum progression free interval (PPFI) (potentially platinum-sensitive), adequate organ function and evaluable (Rustin and/or RECIST) disease. Standard anti-emetic prophylaxis and hydration (2L NS with $NaHCO_3$) were given before and after 1-hour ProLindac™ infusion.

Results: From June 2006 until August 2007, 16 pts were enrolled. Six evaluable pts were treated at each of the first two dose levels (DL0: 900 mg/m²/d; DI: 300 mg/m²/wk; DL+1:1400 mg/m²/d; DI: 466 mg/m²/wk, both q3w). Median age: 64 years (range: 45-68), median number of previous CT lines: 3 (2-3), and median PPFI is 8 months (5.8-41). Median Ca125 levels at baseline were 16.9x upper normal limit (UNL) for DL0 and 4.0 UNL for DL+1pts. Median number of cycles was ≥ 3.5 (2-5) for DL+1 (2 pts ongoing) and only 2 (2-4) for DL0, since 5/6 pts had outright progression. However, 4/6 patients in DL+1 have consistent Ca125 reductions around 30% (15-42%) and had stable disease for a median of 71+ days (one ongoing). Safety: there was no renal toxicity, despite 2/12 pts having creatinine clearance <60 $mL.min^{-1}$ at baseline. Grade 2-3 brief (<5 days) neutropenia and grade 2 anemia were observed in 2/6 pts and 1/6 pts at DL+1 and DL0 respectively. A grade 4 allergic reaction occurred in 1/12 pts after 5 cycles. No acute or cumulative neurotoxicity has been observed. Accrual is ongoing at DL+1: 900 mg/m²/d1 q2w; DI: 450 mg/m²/wk. Additional dose levels (20% DI increase each) will be added in both schedules.

Conclusions: ProLindac™ can be given at DI ≥ 466 mg/m²/wk in q3wks single administration with standard hydration and anti-emetics. Preliminary evidence of consistent anti-tumour activity was observed at this DI in borderline platinum-sensitive OC pts. The confirmation of a large therapeutic index will lead to: a) completion of the phase II part of the trial once RD is achieved and b) the start of ProLindac™ phase I/II combination trials with taxanes, gemcitabine and fluoropyrimidines, by end of 2007. Updated results will be presented.

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