224 POSTER

Zoledronic acid (Zometa), a new potent bisphosphonate, reverses mechanical allodynia and limb sparing in a rat model of bone cancer pain

K. Walker[1], S. Medhurst[1], M. Muller[2], M. Bowes[1], M. Hattenberger[2], T. O'Reilly[2], B. Kidd[1], L. Urban[1], J.R. Green[2]. [1]*Novartis Institute of Medical Sciences, London, UK;* [2]*Novartis Pharma Research, Basel, Switzerland*

Purpose: Severe pain is one of the most common complications of metastatic bone disease. Zoledronic acid (ZOL) is a novel bisphosphonate with an imidazole substituent, currently in phase 3 clinical development for the treatment of both malignant bone disease and osteoporosis. ZOL has previously been shown to potently inhibit osteoclastic bone resorption, lytic bone metastases and angiogenesis in preclinical models. This present study investigated the effect of ZOL on pain in a new rat model of bone cancer pain.

Methods: Adult female rats were given intra-tibial injections of MRMT-1 rat mammary gland carcinoma cells (3 ul, 10 million cells/ml). These animals gradually develop mechanical hyperalgesia, mechanical allodynia (skin hypersensitivity to non-noxious stimuli) and hind limb sparing, beginning on day 12-14 following cell injection. ZOL was administered 3 times a week (10 and 30 ug/kg s.c.) from the day of cell injection, allodynia and limb sparing were then repeatedly measured for up to 21 days.

Results: ZOL produced a profound inhibition of hind limb sparing and mechanical allodynia. In comparison to vehicle-treated controls, which showed maximal hind limb sparing by day 19, rats given the higher ZOL dose did not develop any sign of hind limb sparing over 19 days following intra-tibial cell injection. However, when administered as a single injection (100 ug/kg, s.c.) on day 19, ZOL had no acute effect. By contrast, acute treatment with morphine (1-10 mg/kg, s.c.) produced a dose-dependent reduction in mechanical allodynia and, at the highest dose only, also a significant reduction in hind limb sparing.

Conclusion: Alleviation of bone pain is a frequent clinical observation in cancer patients treated with bisphosphonates but the mechanism of action remains unclear. This new rat model should facilitate further studies on the pathophysiology of bone cancer pain and its amelioration by bisphosphonates. The marked beneficial effects observed with ZOL in the rat model indicate that the compound has the potential to reduce pain in cancer patients with bone metastases.

225 POSTER

Functional spectrum of an HPTLC analysis station in a hospital pharmacy quality assurance program

A. Paci, J.-B. Rey, S. Demirdjian, P. Bourget. *Gustave-Roussy Institute, Clinical Pharmacy, Villejuif, France*

Purpose: As a part of the development of a quality-assurance program (QAP), an analytical platform was installed in the Department of Clinical Pharmacy. This platform consists in two analytical units: the first one uses high-performance thin-layer chromatography (HPLTC) combined with densitometry while the second one uses high-performance liquid chromatography (HPLC) combined with the most common detection modes and a powerful liquid chromatography-tandem mass spectrometry (LC/MS/MS) for structural analysis.

Methods: The HPTLC-CAMAG® consists in: an HPTLC-vario® development chamber, 2) TCL Sampler III® automated applicators, 3) teflon migration chambers and 4) a TLC Scanner 3® densitometer controlled by CATS 4® software. HPTLC allows to obtain chromatograms in 10 min, and run over a migration pathway of 5-6 cm. The plates are read by absorption-reflection or fluorescence-reflection at an ad hoc wavelength (190-800 nm). The peaks areas scanned are measured by the trapezoid method. The calibration curves are generated by Michaelis-Menten non-linear regression, and validated by internal quality control. The analytical yield is high, i.e., up to 50 assays and 250 determinations per day. HPTLC analysis covers a wide functional range; 1) as a teaching tool for separative analysis and GLP, 2) it is an invaluable method for the optimisation of mobile phases and for the determination of absorption spectra and absorption maxima, with the aim to developing HPLC methods in complex matrices, 3) it provides major support for post-production quality control of prescribed hospital preparations of all types e.g. those connected with narcotic analgesia (morphine, fentanyl, sufentanyl...), and off course chemotherapy (MTX, 5-FU, Ara-C, dFdC, CDDP, LOHP, ADM, DRB, 4-EPI, IDA, VP16, IFM, CPM). Furthermore, it can also be used for dry dosage analysis, 4) it is an useful tool in pharmaceutical assessment e.g., in studies on the substances physico-chemical characteristics (identity, purity, concentration, stability and compatibility) particularly with regards to generic products and 5) it can contribute to monitoring the container-content interactions.

Conclusion: HPTLC quantitative and qualitative analysis has now reached a remarkably high level of development and performance. After 30 months in operation and 25, 000 assays, the HPTLC analysis unit has become one of the mainstays of the Gustave-Roussy QAP with a cost of no more than 1.5 US$ per routine assay.

226 POSTER

Phase I-II study: clinical and pharmacokinetic data of docetaxel (DTX), carboplatin (CBDCA) and concomitant radiotherapy (RT) in stage IV head and neck cancer

M. Airoldi[1], S. Marchionatti[1], F. Pedani[1], M. Danova[2], V. Recalenda[3], L. Infante[4], E. Cagliero[4], L. Cattel[4]. [1]*S.Giovanni A.S. Hospital, Medical Oncology, Turin, Italy;* [2]*University of Pavia, Inst. of Internal Medicine and Oncology, Pavia, Italy;* [3]*ASL 19, Service of Pharmaceutical Assiatance, Asti, Italy;* [4]*University of Turin, Postgraduate School in Clinical Pharmacy, Turin, Italy*

Purpose: In vitro studies showed that DTX and CBDCA combination enhances the effects of radiation more effectively than either drug separately. We investigated the pharmacokinetics of these drugs administered at low doses as radiosensitizer concomitant with hyperfractionated RT. Toxic effects and clinical efficacy were also studied.

Methods: 30 patients with unresectable stage IV squamous-cell head and neck cancer received RT (70Gy over 7 weeks, 5 days weekly) concurrent with CBDCA AUC 0,40min·mg/ml (20' i.v. infusion) from day 1 to 5 of weeks 1, 3, 5, 7 and DTX 30mg/m2 (1h i.v. infusion) on days 10, 24, 38. Site of disease: oropharynx, 14 patients; hypopharinx, 8; oral cavity, 5; larynx, 3. Nodal stage: N1, 2; N2, 18; N3, 10. In 11 patients a pharmacokinetic evaluation was done. Several blood samples were collected during and after anticancer agents administration;. The CBDCA plasma concentration was measured as ultrafiltrable free platinum by FAAS. We used HPLC analysis for DTX determination.

Results: The Cmax plasma levels of CBDCA ranged from 3361-2044 ng/ml (at 20') whereas Cmin was reached 5 h after i.v. administration. On 10thday mean DTX Cmax was 735 ng/ml, AUC 0,05min·mg/ml, clearance 65,14 l/h. At the end of locoregional treatment we had 22 CRs (73%) and 8 PRs (27%). After surgical salvage, the number of CRs increased to 24 (80%). Mucosal toxicity (grade III-IV in 21 patients = 70%) was the main dose-limiting toxicity. Grade III dermatitis and leukopenia was observed in 13 (43%) and 11 (36%) patients respectively.

Conclusions: CBDCA+DTX and concomitant RT is feasible and effective treatment in locally advanced head and neck cancer; acute mucosal and cutaneous toxicity were frequently severe but manageable. Concurrent CBDCA, DTX and RT do not alter pharmacokinetic drug behaviour respect single-agent data. During the first course of chemotherapy, the experimental AUC values and those calculated by Calvert formula were similar. However, during following weeks a progressive increase of platinum levels was observed. It could be assumed that a bias in dose calculation occurred because a non linear relationship between creatinine clearance and CBDCA clearance. Further investigation is needed to establish more clearly the significance of variation in free and bound Pt repeated courses of CBDCA administration.

227 POSTER

Feasibility of combination of cisplatin (CDDP) and gemcitabine (GEM) in non-small cell lung cancer and other solid tumors: analysis of dose-intensity (DI) and compliance of four different schedules

S.P. Hector, R. Cavina, A. Sala, F. Latteri, E. Campagnoli, V. Ginanni, E. Morenghi, L. Rimassa, G. Biancofiore, A. Santoro. *Istituto Clinico Humanitas, Medical Oncology and Hematology, Rozzano-Milano, Italy*

The combination of CDDP plus GEM is active and improves survival in advanced NSCLC, but the optimal doses and timing to deliver both drugs is not yet well defined. Therefore, we analyzed the DI, toxicity and activity of four different schedules of CDDP and GEM in three different consecutive studies performed in our institution. Untreated patients with stage IIIA-B/IV NSCLC, ECOG PS < 2, adequate bone marrow, liver and renal function were enrolled. Toxicity was evaluated according to the SWOG criteria and DI was calculated with the method described by Hryniuk, as mgs per sqm per week during the whole treatment (day 1 of the first cycle to the last cycle day). Treatment programs were as follows: A) CDDP 70 mgs/sqm d2 + GEM 1000 mgs/sqm d1,8,15 q4W; B) CDDP 70 mgs/sqm d2 +

GEM 1000 mgs/sqm d 1,8 q3W; C) CDDP 70 mgs/sqm d2 + GEM 1000 mgs/sqm d1,4 q3W; D) CDDP 80 mgs/sqm d1 + GEM 1250 mgs/sqm g1,8 q3W. Programmed dose-intensity (PDI), received dose intensity (RDI) and toxicities were evaluated. Results were as follows: No.pts/cycles A:54/194, B:53/204, C: 36/133, D 12/45; CDDP PDI/RDI A:17.5/16.4, B:23.3/21.4, C:23.3/21.2, D:26.6/21.4; GEM PDI/RDI A:750/592, B:666/589, C:666/610, D:833/555; Grade III-IV neutropenia A:22%, B:28, C:8, D:26; Grade III-IV PLT A:30%, B:5, C:2, D:20; Grade III-IV anemia A:2%, B:9, C:1, D:9.

In conclusion: 1) despite the higher PDI of D, RDI of GEM was the lowest; 2) although the higher PDI of GEM of the q4W regimen, the q3W schedules B and C had similar RDI of GEM; 3) The modified q3W schedule C yields a valid alternative for treatment because of its better toxicity and higher RDI of GEM. According to these results the use of the four-day schedule appears highly advisable.

228 POSTER

Stability assessment of CPT11 and LOHP in hyperthermic intraperitoneal chemotherapy

C. Bouteiller[1], A. Paci[1], L. Mercier[1], D. Elias[2], P. Bourget[1]. [1] *Institut Gustave-Roussy, Clinical Pharmacy, Villejuif, France;* [2] *Institut Gustave-Roussy, Oncological Surgery, Villejuif, France*

Purpose: In the treatment of peritoneal carcinomatosis, hyperthermic intraperitoneal chemotherapy (HIPC) uses platin derivatives in association with another cytostatic agent. This study deals with the possibility to use oxaliplatine (LOHP) and irinotecan (CPT11) simultaneously in hypotonic or isotonic solutions for HIPC treatment.

Methods: HIPC consists the administration of chemotherapy in intraperitoneal cavity during 30 minutes at 41-43°C. LOHP and CPT11 were prepared in different iso- (dextrose 5%, 300 mOsmol/kg) and hypo-osmolar solutions (200, 150, and 100 mOsmol/kg). High-Performance Thin-Layer Chromatography (HPTLC), with automatic sampler ATS® III, horizontal developing chamber and spectrophotodensitometer TLC Scanner III Camag®, was used for analytical measurement (identity, purity and concentration). Calibration range were studied between 100 and 800 μg/mL for LOHP and between 100 and 1000 μg/mL for CPT11 and validated by two QCs. Stability study was carried out regarding different parameters such as concentration (high level:1400 for LOHP, 1800 μg/mL for CPT11 and low level: 250 and 600 μg/mL, respectively), temperature (20°C vs 50°C), -contact-time (t0, 1 and 4 hours). The dilution of the anticancer drugs were made in water for the blank point and in iso- and hypo-osmolar dextrose solutions. The osmolality of each solution were assessed with an automatic osmometer Roebling®.

Results: The stability study showed a decrease in LOHP amounts between 15 to 20% when CPT11 is added, independently to the temperature and the contact-time. No degradation was put in evidence for CPT11 whatever the conditions: 1) solvent, 2) temperature, and 3) contact-time i.e. -2.8 to 0.3% and -1.1 to 1.3% at 20°C and 50°C, respectively. These differences are not statistically significant, according to the method repeatability and reproducibility (respectively, 4.8 and 5.0%).

Conclusion: DichloroDACH platine is known to be a degradation product of LOHP when this latter is used in NaCl 0.9% due to chloride interactions. The fact that CPT11 is marketed as a hydrochloride salt can be the reason of the decrease in the amount of LOHP. In fact, molarity calculations and HPTLC plates seem to confirm the formation of this degradation product (i.e. 20%). Complete identification of degradation product is being carried out using mass spectrometry.

229 POSTER

Pharmaceutical tracking integrated with the patient file; development of a tracking software

P. Bourget[1], V. Barath[1], J. Guntz[2], J. Bourgain[3], M. Legros[1], S. Demirdjian[1]. [1] *Institut Gustave-Roussy, Department of Clinical Pharmacy, Villejuif, FRANCE;* [2] *Institut Gustave-Roussy, Direction of Information Systems, Villejuif, FRANCE;* [3] *Institut Gustave-Roussy, Department of Anesthesia, Analgesia, Intensive Care, Villejuif, FRANCE*

The Department of Clinical Pharmacy (DCP) of Gustave Roussy Institute, has developed a tracking software package integrated with the patient file. The Tracking & Medical Devices Unit manages the Blood Derivative Medicinal Products tracking circuits, the circuits of more than 400 Sterile Medical Devices and, generally speaking, those for all pharmaceutical goods for which tracking is compulsory. SIMBAD-TRACE® software package has been developed in situ and was first open for access in March 1999. It enables pharmaceutical tracking data to be accessed from 500 networked workstations. The tracked references generated approximately 15,000 movements in 2000. In terms of performance, the system achieves 3 complementary objectives: 1) reporting tracking scores which reflect the ability of both DCP and the hospital to pertinently respond to a complex regulatory requirement on a daily basis, 2) the contribution of the tool to cost containment with respect to allocating rare goods and, 3) the contribution of the software package to the implementation of medical devices vigilance inquiries, particularly the descending ones. Finally, after 2 years in operation, SIMBAD-TRACE® has became one of the pillars of our Quality Assurance Program: tracking scores appeared to be, during this period, between 98 and 100% for the sterile medical devices tracked

Drug resistance

230 POSTER

GST genetic polymorphisms and disease outcome after platinum/paclitaxel based chemotherapy in advanced ovarian carcinoma

R. Medeiros, M. Freitas-Silva, N. Afonso, C. Palmeira, C. Afonso-Lopes, A. Vasconcelos, M. Osorio, C. Lopes, D. Pereira. [1] *Instituto Portugues de Oncologia, Porto, Portugal*

The glutathione S-transferases (GSTs) are a group of multifunctional enzymes that catalyze the conjugation of glutathione with cytotoxic agents. GSTs genetic polymorphism with a homozygous deletion (null genotype) of the genes lead to the absence of the enzyme. In the present study we analysed GSTM1 polymorphisms in the genomic DNA isolated from peripheral blood of 24 patients with ovarian cancer treated with chemotherapy (paclitaxel and cisplatinum) after surgery. The median follow-up period of all patients was 27 months. For the disease free interval we found that for the group of cases with GSTM1 wild-type the median time was 22 months and that all cases of GSTM1 null genotypes were disease free at the end of the follow-up. This differences were statistically significant by the log rank test ($p= 0.02$). The caracterization of the drug metabolizing genetic individual profile, can be of great interest in clinical oncology, for the definition of the optimal chemotherapy for each patient, improving eficiency and reducing drug toxicity and poor drug responses.

We gratefully acknowledge funding of this work by the MInistry of Health of Portugal (CFICS- 218/99).

231 POSTER

Inhibition of heat shock protein 27 (HSP27) by paclitaxel reduce cisplatin resistance

H. Tanaka, F. Keiichi, T. Yasumasa, M. Kenichiro, K. Ichiro. *Kawasaki Medical School, Obstetrics and Gynecology, Kurasiki-City, Japan*

Background and Aim: It is known that heat shock protein 27 (HSP27) expression correlates with chemotherapeutic resistance. In a previous study, we reported that paclitaxel (P) treatment suppressed the expression of HSP27. Although combination chemotherapy with P and cisplatin (C) has been demonstrated to clinically improve a patients prognosis, the mechanism underlying this clinical observation remains unclear. The aim of this study was to investigate whether inhibition of HSP27 expression by P can reduce C resistance.

Method: C-sensitive and resistant HeLa cells were treated with C and P. The treatment sequences examined were C-to-P (CTP) and P-to-C (PTC). Each cell was treated for 48 hours with each agent at 50% of the inhibitory concentration (IC50). The cytotoxic effect was evaluated by the MTT assay. Staining for HSP27 expression was done using the indirect immunofluorescence technique. Then expression was analyzed with a flow cytometer and comparing the relative ratio of the fluorescent intensity with that of sensitive cells at a non-treatment level of 1.0. The relation between HSP27 expression and the cytotoxic effect with regard to the contact sequence of the therapeutic drugs was studied.

Results: As for the cells receiving combined treatment with C and P, the surviving fraction of sensitive cells after CTP was 51% and PTC, 31%. The surviving fraction of resistant cells after CTP was 47% and PTC, 26%. These results indicate that PTC treatment had the strongest cytotoxic effect on both sensitive and resistant cells. Relative expression of HSP27 was not different between the sensitive cells (1.0) and resistant cells (1.1). C treatment induced a HSP27 expression in both sensitive cells (1.8) and resistant cells (2.0), however, HSP27 expression was not observed in either of them 0.9 and 1.0, respectively, when treated with P.