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.

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Stability assessment of CPT11 and LOHP in hyperthermic intraperitoneal chemotherapy

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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.

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

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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

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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.

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

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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.

As for the combination treatment, pretreatment by P inhibited the induction of C-induced HSP27 expression in both C-sensitive and resistant cells. Relative HSP27 expression for the PTC sequence was 1.3 for sensitive cells and 1.1 for resistant cells, whereas that for the CTP sequence was 1.9 for sensitive cells and 2.4 for resistant cells.

Conclusion: Pre-treatment by P appeared to strengthen the cytotoxic effect of C by inhibiting HSP27 expression in both C-sensitive and resistant cells. This observation suggested that inhibition of HSP27 by P play an important role in reduction of the C resistance.

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Screening of beta tubulin mutations in serous and clear cell ovarian carcinoma

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Purpose: Beta tubulin mutations have been implicated in resistance to chemotherapeutic treatment with paclitaxel. The aim of the present study was to screen for beta tubulin mutations in a group of serous and clear cell ovarian carcinoma patients treated with paclitaxel/cisplatin combination.

Methods: We selected 34 ovarian carcinoma patients (26 serous ovarian carcinomas and 8 clear cell ovarian carcinomas), classified as invasive or borderline, treated after surgery with paclitaxel and cisplatinum, for at least 4 cycles. These patients were radio and chemotherapy naïve. DNA was extracted from paraffin embedded tumours, after microdissection, amplified with specific primers for exon 4 of beta tubulin gene, and finally sequenced. 10 patients (29,4%) were chemo-resistant.

Results: None of the cases revealed beta tubulin mutations.

Conclusion: In the studied ovarian carcinoma patients the paclitaxel resistance mechanism was not associated with exon 4 of beta tubulin gene mutations.

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Daunomycin-polypetide conjugates: in vitro antitumour effect in sensitive and multidrug resistant cell lines

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Purpose: Anthracyclines, like daunomycin (Dau) are widely used in the therapy of cancer. Side effects (e.g. immunosuppression, cardiotoxicity) and multidrug resistance developed during the treatment, seriously limit their therapeutic efficiency. Earlier results from our group have demonstrated that coupling of daunomycin with an acid labile spacer (cAD) to an amphoteric branched chain polypeptide, EAK, significantly increased the survival of L1210 bearing mice (1). cAD-EAK conjugate contains two isomers of the cAD, namely a-and b-cis-aconytil-daunomycin. Since isomers might have different biological effects, we have prepared both isomers and their polypeptide derivatives. The aim of these studies was to compare their antitumour effect in vitro.

Methods: The in vitro antitumour effect of isomers and isomer containing conjugates was investigated in the following tumour cell lines: C26H colon carcinoma, MDA-MB 435P breast carcinoma, HL-60/sensitive and HL-60/MDR1-HL-60/MRP1 resistant human leukemia cell lines. In HL-60/sensitive, as well as in HL-60/MDR1 and HL-60/MRP1 resistant cell lines, we have compared the uptake and effect of daunomycin with those of cAD-EAK conjugate, since daunomycin is the substrate of the MDR1 and MRP1 membrane proteins.

Results, Conclusion: Data suggest that one of the isomer (cAD1, IC50~5 μmol; cAD2, IC50~250 μmol) and one isomer-conjugate (cAD1-EAK, IC50~8 μmol; cAD2-EAK, IC50~300 μmol) was more effective in tumour cell lines studied. In addition we have observed that the cAD-EAK polypeptide conjugate enters not only the sensitive, but also the MDR resistant human leukemia cells.

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Pharmacokinetics

234 POSTER

Ecteinascidin (ET-743) pharmacokinetics(PK) -overview of phase i and advanced phase II results

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ET-743 derives from the tunicate Ecteinascidia turbinata. It showed activity during Phase I and II in several tumor types, such as sarcoma, breast and ovary. Its potency (IC50 pM-low nM) required a sensitive analytical method, liquid chromatography-tandem mass spectrometry (LC-MS/MS). The dose-limiting toxicity (DLT) is myelosuppression. Other toxicities include transaminitis, increased alkaline phosphatase (AP) or bilirubin (bil) and rare (0.9%) cases of rhabdomyolysis. ET-743 is eliminated by the liver, probably by metabolism by CYP3A4 and probably CYP2C9/10 (but not 2C8), with contribution of CYP2E1 and 2D6. Urinary recovery is <2%. ET-743 PK features are: dose linearity after 3 or 24 h infusion, but not 1 or 72 h infusion, wide distribution (Vss 800-5500 L/m2). slow elimination (t1/2 40-300 h) and high clearance (Cl) (mean values at the recommended dose (RD) 22-50 L/(hr*m2) -50% to 90% of expected liver blood flow-). Most profiles after 24 h infusion are best fit by a 2-compartment model (median (M) half lives alpha and β; 0.5 h and 88.8 in Phase I patients (pts); 0.5 and 45.6 h in Phase II pts). In single stage population PK (NONMEM) the best fit is provided by a 3-compartment model (M half lives: alpha 0.3 h, β 2.6 h, gamma 135.9 h). After 3-h infusion, most profiles are best fitted by a 3-compartment model (M half lives: alpha 0.2 h, β 2.2 h, gamma 67.6 h). ET-743 toxicity is exposure related. AUC is the only PK parameter predictive for hematological toxicity in the 24 h schedule. In the 3 h schedule Cmax is predictive too. The proportion of treatment courses with DLT increases from 8.5% to 40% (p=0.009) when comparing courses with AUCs≤ and >70 h*mcg/L in the 24 h schedule. The pts with rhabdomyolysis had AUCs >100 h*mcg/L. AUC in responding and non-responding pts were similar: M(range) 41.6(17.1-76.4 h*mcg/L) in responders vs. 38.8(16.7-178.8 h*mcg/L) in the other Phase II sarcoma pts treated with 24 h infusion. Biliary function is determinant for ET-743 PK. The best predictive parameter is increased AP (at baseline or during treatment). ET-743 Cl decreases by 50% with relatively low increases in AP (10% over the upper limit of normality). Bil increases occur less frequently but cause a greater decrease in Cl. Colorectal cancer pts had decreased ET-743 Cl; mean(SD) 20.7(6.8) in colorectal vs. 33.0(21.3) L/h*m2; p=0.006 in Phase II sarcoma pts treated with ET-743 as a 3-h infusion. ET-743 PK offers opportunities to optimize its therapeutic range.

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Pharmacokinetics (PK) of irofulven using three different intermittent 5-minutes (MIN) infusion dosing schedules (sch) in advanced solid tumors (AST): Final results

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Irofulven, an acylfulvene analog of illudin S, has shown promising anti-tumor activity during preclinical/clinical development, with delayed thrombocytopenia (T), asthenia and nausea/vomiting as treatment-limiting toxicities. A new phase I study exploring 3 different intermittent dosing sch with a 5-min infusion (A: D1–8–15 q4 weeks (w), B: D1–8 q3w, C: D1–15 q4w) is ongoing in patients (pts) with AST. Starting dose intensity (DI) was 10 mg/m^2/w (i.e. 13.3, 16 and 18 mg/m^2/dose in sch A, B, C respectively). Planned DI (PDI)

Dose (mg/m^2)	N eval. 1 pts-cycle 1	D1 Cmax (ng/ml)	AUC (ng/mlxh)	Clt (l/h/m^2)	$T_{1/2}$ beta (min)
13.3	5	190 ± 99	25.1 ± 12.8	654 ± 319	5.6 ± 1.8
15	3	211 ± 30	29.1 ± 4.4	522 ± 72	5.6 ± 2.9
16	7	300 ± 307	38.9 ± 38.4	855 ± 750	5.2 ± 2.5
18	8	306 ± 160	36.4 ± 17.0	645 ± 424	4.3 ± 1.9
18.6	6	413 ± 335	45.8 ± 27.2	509 ± 240	3.7 ± 1.4
20	2	334 ± 74	60.0 ± 10.3	339 ± 59	4.1 ± 1.8
21	6	599 ± 345	66.1 ± 38.6	493 ± 423	6.6 ± 2.1
24	6	586 ± 562	78.5 ± 68.4	431 ± 186	4.1 ± 1.9
28	7	754 ± 268	92.2 ± 48.7	380 ± 174	6.0 ± 1.2