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.

232 POSTER

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.

233 POSTER

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.

235 POSTER

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