Material and Methods: Plasma and urine concentrations of LC and ALC from eleven patients under carboplatin chemotherapy (1 hr intravenous infusion; AUC dose of 4.8±1.1 mg ml^{-1} min^{-1}) were determined before, during and after treatment using a high performance liquid chromatography method.
Results: Before carboplatin chemotherapy, the plasma concentration (mean±SD) of LC and ALC was 47.8±10.9 and 7.0±1.0 nmol/ml, respectively, and remained constant for the entire period of plasma collection. In contrast, the urinary excretion of LC and ALC, increased significantly during the chemotherapy from 115±105 to 480±348 μmoles/day ($p < 0.01$; One-way repeated measures ANOVA) and from 41±41 to 89±52 μmoles/day ($p < 0.05$) for LC and ALC, respectively. It normalised six days after the end of chemotherapy. Similarly, the renal clearance of LC and ALC increased substantially during the chemotherapy from 1.67±1.43 to 9.05±9.52 ml/min ($p < 0.05$) and from 4.02±4.51 to 7.97±5.05 ml/min (p = not significant) for LC and ALC, respectively, reaching normal values six days after the end of chemotherapy. However, plasma concentration and urinary excretion of glucose, phosphate and nitrogen and the creatinine clearance were not affect by carboplatin treatment indicating no impaired function of the kidney.
Conclusions: Treatment with carboplatin was associated with a marked urinary loss of LC and ALC, most likely due to inhibition of LC (and ALC) reabsorption in the kidney.

1464 POSTER

Enhancement of gemcitabine antitumor effects by pretreatment of S-1, a novel oral derivative of 5-fluorouracil, in pancreatic cancer

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Background: Although Gemcitabine (GEM) has been accepted as a key drug for treatment of pancreatic cancer patients, its efficacy as a single agent remains to be unsatisfied. On the other hand, S-1 is a novel oral derivative of 5-FU prodrug tegafur combined with two modulators, 5-chloro-2, 4-dihydropridine and potassium oxonate, and S-1 has the promising antitumor effect against various malignant tumors including pancreatic cancer. It was recently reported that cellular uptake of GEM was mediated by the equilibrative nucleoside transporter 1 (ENT1) and ENT1 expression was enhanced by TS inhibitor such as 5-fluorouracil (5-FU). In this study, to enhance the effect of GEM by S-1, we evaluated the antitumor effects and treatment schedules of combination therapy with GEM and S-1 in *in vivo* pancreatic cancer model.
Material and Methods: Expression of ENT1 was determined by quantitative RT-PCR. GEM cellular uptake was determined using [3H] GEM. Seven pancreatic cancer cell lines (AsPC1, BxPC3, MiaPaCa-2, PSN1, Panc1, PCI6, and KMP4) were treated *in vitro* with 5-FU either before or following exposure to GEM. Growth inhibitory effects *in vitro* were determined by MTT assay. Human pancreatic cancer xenografts (BALB/c nu/nu mice) were prepared with subcutaneous injection of MiaPaCa-2 cells, and divided into the following six groups (5 mice /group): no treatment; weekly intraperitoneal injections of GEM (240 mg/kg); daily oral administrations of S-1 (10 mg/kg/day) for 5 consecutive days a week; sequential combination treatment with GEM prior to S-1; coadministrations of GEM and S-1; sequential combination treatment with S-1 prior to GEM. The antitumor effects were evaluated with the tumor volume at the day 18.
Results: Significant increases in ENT1 expression and GEM cellular uptake were observed after 5-FU treatment *in vitro* and S-1 treatment *in vivo*. The *in vitro* growth inhibitory effect was significantly greater in the sequential treatment of 5-FU followed by GEM in all cell lines except for Panc1. Furthermore, the significant tumor growth inhibition *in vivo* was observed in the mice treated with S-1 followed by GEM compared with either untreated mice or the mice treated with gemcitabine followed by S-1.
Conclusions: The administration of gemcitabine followed by S-1 provides greater inhibitory effects than the other GEM/ S-1 schedules. These data suggest new effective combination treatment for patients with pancreatic cancer.

1465 POSTER

A heparan-mimetic protects mice from radiation-induced mucositis

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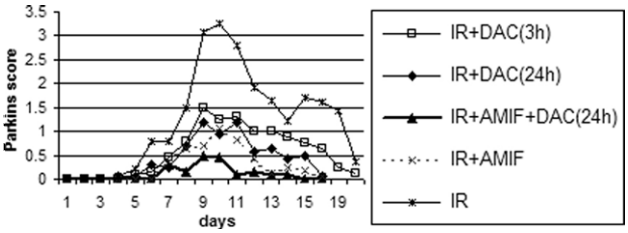
Background: The purpose was to evaluate the effect of RGTA-DAC (ReGeneraTing Agent), a synthetic heparan-mimetic, on radiation-induced mucositis in a murine model and on tumor growth *in vivo* and *in vitro*.
Material and methods: Reagents: RGTA-DAC 1mg/kg for intra-peritoneal (IP) injection; 10 μg/ml in spray solution; 10 μg/ml for *in vitro* assay. Amifostine (Ethyol®) 200 mg/kg for IP injection.
Radiation-induced mucositis: the oral region of C57 black female mice was selectively irradiated with a single dose of 16.5 Gy; mucosal reactions were evaluated daily for 21 days and scored by the Parkins scoring system (Parkins et al, 1983).
Tumor growth: 3×10^6 HEP2 cells (human pharynx cancer cell line) were implanted subcutaneously into nude Balb/c mice. Animals were irradiated with a single dose of 15 Gy selectively to the tumor when tumor diameter reached a mean of 5 mm. The tumor volume was evaluated for 21 days by the formula: length × $width^2/2$ and tumoral response was estimated versus initial volume.
Clonogenic survival: survival fraction of HEP2 cells was evaluated using the Park model; cells were exposed to irradiation (IR) at 0, 2, 4, 6 Gy and to RGTA-DAC 2 h after IR.
Results: Radiation-induced mucositis scored after IR plus RGTA-DAC spray followed by IP injections 3 h after, at day 1 and every 3 days, was significantly lower than after IR alone ($p < 0.001$). A marked decrease of severity and duration of mucositis was observed with administration of RGTA-DAC 3 h after IR ($p = 0.0006$) and 24 h after IR ($p = 0.001$) compared to IR alone. Association of amifostine 10 min before IR with RGTA-DAC 24 h after IR evidenced a major and better protection than RGTA-DAC 3 h or 24 h after IR ($p = 0.002$; $p = 0.001$) and than amifostine 10 min before IR ($p = 0.005$).
Tumor growth: administration of RGTA-DAC IP 3h after IR, then at day 1 and every 3 days didn't evidence significant interference on tumor growth associated (p = NS) or not (p = NS) to IR.
Clonogenic survival: the assay of radio-sensitivity *in vitro* didn't show a significative activity of RGTA-DAC on HEP2 cells clonogenicity (p = NS).



Mucositis

Conclusions: RGTA-DAC demonstrated a protective activity in radiation-induced mucositis in mice, without interference on tumor growth; RGTA-DAC associated with amifostine gave an almost total protection. Further investigations are neeed to understand the selective protective activity on healthy tissues.

1466 POSTER

EGFR and C-KIT/CD117 gene mutational screening and oncoprotein expression in patients with cancer of unknown primary: Implications for molecular pathophysiology and therapy

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Background: Cancer of unknown primary site (CUP) is a heterogeneous group of malignancies that often follow an aggressive clinical course. In order to elucidate its biology, we studied the expression of two transmembrane receptor genes with tyrosine kinase activity, C-KIT/CD117

and EGFR, both coding for proteins currently recognized as potential targets of tyrosine kinase inhibitors (TKI).
Methods: Fifty archival tissue specimens from CUP patients were screened for C-KIT and EGFR mutations. All samples were screened for the presence of small deletions and/or insertions in exon 11 coding sequences for the juxtamembrane domain of the C-KIT protein and for polymorphisms of a CA dinucleotide small sequence repeat (SSR) in the untranslated regulatory sequence in intron 1 of the EGFR gene by means of polymerase chain reaction-based single strand conformational polylmorphism (PCR-SSCP). Thirty-six out of 50 samples were stained by immunohistochemistry for EGFR (K1494 DAKO) and C-KIT/CD117 (A 4502 Dako) protein expression.
Results: Among the 35 immunostained specimens, 26 (74%) showed EGFR expression, 4(12%) strong, 15 moderate and 7 weak. In view of previous studies showing an association of decreasing EGFR transcriptional activity with increasing numbers of intron 1 repeats, we determined the SSR length of EGFR intron 1. We detected five alleles with CA repeat numbers 16 to 20. Allele 16 showed the highest frequency (39%) followed by allele 18 (34%), 19 (11%), 20 (11%) and 17 (5%). All samples were heterozygous, the commonest genotype consisting of allele lengths of 16/18 dinucleotides (78%). Seven samples revealed an unexpected genotype of 3 CA –length alleles, probably due to genetic instability, and were associated with EGFR overexpression in 40% of cases. Samples with alleles of $\leqslant$18 and >18 CA repeats showed EGFR overexpression in only 8% and 0% of cases respectively. C-KIT overexpression was found in 2 specimens (6%) while 7 showed moderate and 22 weak staining. No gain of function mutations nor any polymorphism were found by PCR-SSCP in C-KIT exon 11.
Conclusions: Positive EGFR and C-KIT/CD117 expression is seen in the majority of CUP patients, while overexpression less often. Our findings indicate the presence of an association between EGFR protein overexpression and the allelic length of EGFR intron 1 CA SSR. This observation may provide clues for the molecular biology of the disease and holds promise for TKI-based therapeutic interventions. C-KIT oncoprotein expression is not associated with activating mutations in exon 11 of the gene, indicating that such mutations are not implicated in CUP molecular pathophysiology.

1467 POSTER

RAD51: A DNA repair target for increasing the therapeutic potential in bladder cancer

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Background: Organ preservation using combination chemoradiation with gemcitabine or cisplatin has become an increasingly important strategy in the treatment of bladder cancer with complete response rates of ~70% in selected patients. Chemoresistance has been linked to the overexpression of RAD51, a pivotal protein in homologous recombination (HR), a DNA double strand (DNA-dsb) repair pathway. Therapeutic manipulation of RAD51 expression, or RAD51 signaling pathways, may allow increased tumour cell kill following ionizing radiation (IR) within a favorable therapeutic ratio.
We hypothesize that manipulation of the HR-RAD51 pathway could lead to a therapeutic gain for bladder cancers treated with XRT-cisplatin or XRT-gemcitabine.
Materials and Methods: Using the RT112 bladder-TCC cell model, clonogenic survival is being determined for cell exposure to IR, cisplatin(cDDP), gemcitabine, and mitomycin C (MMC; the latter used to measure relative HR). Imatinib, an inhibitor of c-ABL/RAD51 signaling, was used at a variety of doses from 7.5–50 μM alone, and in combination, with IR, gemcitabine and cDDP to look for supra-additive effects. Protein expression for c-ABL and DNA-dsb-related protein expression is determined using Western blots. Use of a plasmid-based HR assay (DR-GFP construct) within a RT112 clone will enable functional assessment of HR following imatinib treatment.
Results: Decreased RAD51, but not KU70, protein expression was observed at 24hours following 50 μM imatinib in RT112 cells. The ICD50 for gemcitabine is 13 nM and for MMC is 75 nM for RT112 cells. SF2 Gy following IR for RT112 cells is 0.68. Addition of imatinib resulted in an 8-fold increase in cell kill at 2 Gy (ratio cell kill 2 Gy: 2 Gy+gleevec = 1: 8.4). Ongoing experiments are testing for similar chemosensitization. Transfection of DR-GFP into RT112 cells was successful and will be used to correlate HR levels pre- and post-imatinib to cell toxicity in both RT112 cells and normal fibroblasts.
Conclusions: RAD51 inhibition may be a novel strategy to increase the radiosensitization of bladder cancer if it translates to maximal sensitization of bladder tumour cells over that of normal tissues. This may allow for novel treatment strategies that increase organ-preservation rates and quality of life for bladder cancer patients.

1468 POSTER

A phase 1B, open-label, dose-escalation study of bortezomib (Btz) in combination with gemcitabine (G) and cisplatin (C) in the first-line treatment of patients with advanced solid tumors: preliminary results

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Background: Bortezomib (VELCADE®) is a reversible, specific inhibitor of the 26S proteasome. G/C is a first-line treatment for several advanced solid tumors. MTD and tolerability of a weekly and twice-weekly schedule of Btz in combination with G/C are investigated.
Methods: Chemonaïve patients (pts) (KPS $\geqslant$ 70%) with advanced solid tumors received G 1000 mg/m^2 days 1&8, C 70 mg/m^2 day 1, on a 21-day cycle. In the weekly schedule pts received Btz days 1&8; dose levels (DL): 1.0?1.3?1.6 mg/m^2. In the twice-weekly schedule, pts received Btz days 1, 4, 8&11; DL: 0.7?1.0?1.3 mg/m^2. Safety assessments included CTC 3.0, audiometry and FACT/GOG-NTX. Limited PK analysis is carried out at MTD. Serum is collected for proteomics.
Results: So far, 31 pts (34 needed) were treated for at least 1 cycle: 19 in the weekly schedule (12 pts at 1.0 mg/m^2, 7 pts at 1.3 mg/m^2) and 12 in the twice-weekly schedule (3 pts at 0.7 mg/m^2 and 9 pts at 1.0 mg/m^2). Pt characteristics: Median age: 55; Sex M/F: 21/10; KPS < 80%/$\geqslant$80% = 5/26; tumor types: 24 NSCLC, 4 urothelial cell ca, 1 unknown primary, 1 pancreas ca, 1 HCC. First-cycle DLTs were observed in the weekly schedule at Btz 1.3 mg/m^2: GR3 diarrhea (1 pt), GR4 platelets (plts) (3 pts), GR4 ANC > 5 days (2 pts). In the twice-weekly schedule at Btz 1.0 mg/m^2, 1 pt with DLT was seen: GR4 plts with bleeding, GR4 febrile neutropenia. The MTD of Btz was 1.0 mg/m^2 in both schedules. At MTD, most common GR3/4 toxicities: plts 71%/14% (twice-weekly), 42%/17% (weekly), neutropenia 43%/14% (twice-weekly), 25%/8% (weekly). Treatment was generally well tolerated. No $\geqslant$GR2 drug-induced sensory neuropathies have been observed. Addition of Btz did not appear to lead to additional N/V, or diarrhea compared to G /C alone. C was reduced in 2 pts because of GR2 ototoxicity. There was one case of GR4 left ventricular dysfunction. Subsequent follow-up of following pts by MUGA revealed no marked EF decrease. In the first 25 evaluable pts, there were 11 partial responses (NSCLC, UCC, ACUP; 2 to be confirmed), 12 pts with stable disease and 2 pts with progressive disease. 7 out of 9 confirmed responses were seen in the weekly schedule.
Conclusion: The MTD of Btz combined with G 1000 mg/m^2 and C 70 mg/m^2 is equal in the two schedules at 1.0 mg/m^2. The combination has been well tolerated with less (myelo) toxicity being observed in the weekly regimen. In general, Btz may increase myelosuppression of G/C chemotherapy. Updated information (including PK) at the meeting.

1469 POSTER

Molecular mechanisms of cis-4-hydroxy-L-proline – induced antiproliferative effects in colon and pancreatic tumor cell lines in vitro

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Background: Proline analogs have been supposed to target collagenous proteins in cancer cells ultimately disturbing the formation of extracellular matrix, which directly or indirectly has important functions in the growth and metastasis of solid tumors. The aim of the present study was to investigate the cellular mechanisms affected by cis-4-hydroxy-L-proline (CHP), leading to antiproliferative activity in a panel of tumor cell lines.
Material and methods: Proliferation was assessed using a formazane assay (MTT), EGF receptor expression and cell cycle distribution (propidium iodide) by FACS analysis. Cellular ultrastructure was studied in transmission electron microscopy (TEM).
Results: Application of CHP in concentrations ranging from 50–400 μg/ml resulted in dose-dependent inhibition of proliferation and cell death in a panel of tumor cell lines including lines derived from colon, pancreatic, prostate, breast and other cancers. Tritiated CHP showed uptake by the MeAIB-sensitive proline transporter and was incorporated into cellular proteins. TEM data of CHP-treated cells (Colo 205 and Caco-2 colon lines and BxPC3 and MIAPaCa2 pancreatic cancer lines respectively) revealed the induction of extensive intracellular vacuolization and cell death. CHP-induced ultrastructural alterations were absent in normal fibroblasts and colonocytes. Furthermore CHP was found to induce a pronounced extracellular acidification (0.1–0.4 pH units), not observable upon treatment