For a dog, there was a similar tendency. Biliary excretion continued up to 48 hours after IHL-305 dosing, whereas it was completed at 24 hours after CPT-11 dosing in rats.
Conclusions: After the administration of IHL-305, irinotecan was cleared very slowly from plasma, and most irinotecan in plasma existed as the lactone form. IHL-305 dosing also retained more plasma SN-38 longer than CPT-11 dosing in rats. These pharmacokinetic profiles of IHL-305 were considered to explain its superior antitumor activity other than enhanced permeability and retention (EPR) effect.

154 POSTER

Novel prodrugs of SN38 generated by Multi-Arm Poly(ethylene glycol)

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Background: SN38 (10-hydroxy-7-ethyl-camptothecin) is the active metabolite of CPT-11 (Camptosar®). SN38 has not been used directly as an anticancer drug due to its poor solubility in any pharmaceutically acceptable excipients. Using multi-arm high molecular weight PEG, we have successfully generated novel water soluble prodrugs of SN38.
Material and Methods: In order to increase drug payload, multi-arm PEG was used. In particular, 40k 4-arm-PEG-OH was first converted to PEG acid, then conjugated with a properly protected SN38 intermediate with different amino acid linkers attached to the 20-hydroxyl position to give the PEG-SN38 conjugates. The aqueous stability and hydrolysis property in rat and human plasma were monitored using UV based HPLC methods. The *in vitro* cytotoxicity of all the PEG conjugates was tested in several different tumor cell lines. The *in vitro* metabolism study of PEG-SN38 conjugates was examined in rat hepatocytes.
Results: Using proper protecting and de-protecting strategies, two different chemistries have been developed to synthesize the PEG-SN38 conjugates in high yields. The process was readily adaptable for scale up development. All four PEG-SN38 conjugates had good solubility in water, with up to 4 mg/mL equivalent solubility of SN38 achieved. All compounds showed good stability in saline and other aqueous medium for up to 24 hrs at room temperature. All conjugates demonstrated potent *in vitro* cytotoxicity against a panel of cancer cell lines. The sensitivity of cells to PEG-SN38 was in the order: COLO205 > HT29 = OVCAR-3 > A549. PEG-SN38 conjugates were equipotent to native SN38 and about 10 to 600 fold more potent than CPT-11. PEG-SN38 conjugates were 8 to16 fold more sensitive than Pegamotecan (a PEGylated prodrug of camptothecin) in COLO 205, HT-29 and OVCAR-3 cells. In human plasma, SN38 was steadily released from the PEG conjugates with a doubling time of 22 to 52 minutes and the release appeared to be pH and concentration dependent. Metabolic study using rat hepatocytes showed SN38 released from conjugates formed a phase II SN38-glucuronide metabolite.
Conclusions: Using multi-arm high molecular PEG, we have successfully prepared several water soluble prodrugs of SN38 for direct parental applications. The payload of the parent drug was almost doubled compared to the traditional straight chain PEGylation. High water solubility was achieved. All PEG-SN38 conjugates showed potent *in vitro* anti-tumor activities which are much more potent than the small molecule prodrug CPT-11 and Pegamotecan. These results warrant further study of these conjugates in animals. PEGylation appears to be a promising approach to deliver SN38, a potent but insoluble cytotoxic agent.

155 POSTER

Mass balance, pharmacokinetics and metabolism of [14C] BMS-354825 in healthy male subjects

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Background: Dasatinib (DAS) – a potent, orally active inhibitor of several oncogenic kinases – has demonstrated clinical efficacy in CML and Ph+ ALL. This study assessed the mass balance, PK, metabolism, and routes/extent of elimination of a single oral dose of 100 mg (120 μCi) [^{14}C] DAS in healthy male subjects.
Methods: This was an open-label, non-randomized, single-dose study involving 8 subjects (21−41 y.o.). All received a single oral dose of 100 mg of [^{14}C] DAS solution containing 120 μCi of total radioactivity (TRA). Vital signs, physical exams, ECGs, clinical labs, and adverse events were conducted/monitored. Blood, urine, and feces were collected to measure DAS, the piperazine N-oxide metabolite of DAS (M5) and TRA, and for biotransformation analyses.

	Cmax (ng/mL), GM[a] (CV)	AUC_{∞} (ng h/mL), GM[a] (CV)	AUC_{0-T} (ng h/mL), GM[a] (CV)	Tmax (h), Med[b] (Min, Max)	T-half (h), Mean (SD)	CLR (mL/h), Mean (SD)	UR (%), Mean (SD)
DAS	104.47 (29)	313.97 (42)	298.8 (44)	0.5 (0.25, 1.5)	3.59 (1.01)	404.83 (168.73)	0.12 (0.05)
M5	2.96 (55)	15.28 (53)	8.88 (74)	1.5 (0.75, 3)	3.15 (1.2)	–	1.2 (0.49)
TRA	224.61 (23)	1231.34 (53)	400.43 (41)	0.5 (0.25, 1.5)	3.96 (2.63)	8636.92 (2651.12)	–

[a]GM, geometric mean. [b]Med, median.

In plasma, DAS AUC(INF) accounted for ~29% of the AUC(INF) of TRA. Multiple metabolites were identified with DAS as the major component. In feces, DAS was a prominent component accounting for 19% of the dose. Metabolites M20 (4-hydroxy-chloromethylphenyl DAS) and M6 (the carboxylic acid derivative of DAS) were detected in significant amounts. No conjugated metabolites were detected in feces.
Conclusions: (1) Radioactivity was primarily eliminated in feces. Mean total recoveries through 9 days post dose were 85% in feces and 4% in urine (total mean = 89%). (2) Negligible amounts of DAS and M5 were excreted in the urine, ~1% of dose. (3) The parent drug was an important drug-related component and M5 a minor metabolite in plasma. (4) A single 100 mg dose of [^{14}C] DAS was safe and tolerable.

156 POSTER

Antitumor activity of IHL-305, a novel PEGylated liposome containing irinotecan, in human xenograft models

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Background: Irinotecan hydrochloride (CPT-11) is an antitumor agent that acts by inhibiting DNA topoisomerase I. CPT-11 is widely used in clinic due to its confirmed evidence of antitumor efficacy. IHL-305 is a preparation of irinotecan encapsulated in PEGylated liposome. Liposome preparations are known to be selectively transported to tumor tissues due to the effect of enhanced permeability and retention (EPR). In this study, antitumor efficacy profiles of IHL-305 were evaluated in comparison with CPT-11 using nude mice subcutaneously transplanted with various human cancer cell lines.
Materials and Methods: After transplanting human cancer cell lines (colon, non-small cell lung, small cell lung, prostate, ovarian, and gastric cancer cells) subcutaneously to the inguinal region of nude mice, the animals were grouped on the day when the estimated tumor volume reached about 60–180 mm^3 (Day 0). IHL-305 or CPT-11 was administered intravenously (i.v.) 1–3 times at 4–14 days intervals (total dose 16.875–135 mg/kg or 18.75–270 mg/kg as irinotecan). Physiologic saline or empty liposomes were administered as negative controls with the same administration schedule. Tumors were excised on Day 21, and tumor growth inhibition (TGI) rates (%) were calculated from tumor weights.
Results: The TGI rates for IHL-305 doses (16.875–135 mg/kg) versus CPT-11 doses (18.75–270 mg/kg) tested were 99.2−99.5% vs 35.5−67.2% on QG-56 (NSCLC), 34.7−93.1% vs 4.8−45.8% on NCI-H460 (NSCLC), 86.7−99.8% vs 74.1−88.0% on NCI-H82 (SCLC), 97.9−99.0% vs 48.0−62.3% on PC-3 (prostate), 24.0−89.9% vs 7.7−42.5% on HT-29 (colon), 62.1−91.9% vs 39.0−87.7% on HCT116 (colon), 77.1−80.8% vs 57.3−69.2% on MKN45 (gastric), and 69.1−97.7% 20.2−64.3% on ES-2 (ovarian) cancer xenografts. In all tested xenograft models, IHL-305 demonstrated superior TGI rates to CPT-11 even in HT-29 colon cancer cell line, which has shown intrinsic resistance to CPT-11. No significant changes of body weight were noted in IHL-305 treated groups.
Conclusions: IHL-305 demonstrated stronger tumor growth inhibition effect than CPT-11 on various human cancer xenografts.

157 POSTER

Combination therapy for liver tumor growth and metastasis by low dose rapamycin and FTY720

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Background: Our previous studies demonstrated that the new immunomodulator FTY720 could suppress liver tumor growth and metastasis through down-regulation of cell survival and invasion pathways. On the

other hand, rapamycin shows its anti-angiogenesis function in several cancer therapy. In order to explore the possibility of combination therapy by rapamycin and FTY720, we designed an orthotopic liver tumor nude mice model to investigate the effect of this combination therapy by comparison of the tumor growth and metastases, as well as the related signaling pathways.
Materials and methods
The orthotopic liver tumor nude mice models with different metastatic potential were applied. $5*10^6$ MHCC97H or MHCC97L cells were injected subcutaneously into the right flank of the mice. Once the subcutaneous tumor reached 1 cm in diameter, it was removed and cut into about 1–2 mm cubes which were implanted into the left liver lobe of another group of nude mice. In the single treatment group, rapamycin was given by ip injection at 0.8 mg/kg/3 days. In the combination treatment group, rapamycin (0.5 mg/kg) and FTY720 (2 mg/kg) was administrated by ip injection every 3 days. The treatments were started at 7 days after tumor implantation. The mice were sacrificed at day20, 30 and 40 after treatment, respectively. The tumor growth, proliferation (Ki67), apoptosis (TUNEL) and local/distant metastases were compared among the groups. Hepatic stellate cell activation in the tumor tissue was detected by α-SMA staining. Cell signaling related to invasion, migration (ROCK-Rho) and angiogenesis (VEGF) were compared. The effect of FTY720 and rapamycin on MHCC97H and MHCC97L was also studied in vitro functional tests.
Results
The tumor growth was significantly suppressed by both single and combination treatments by comparison of liver tumor volume. The incidence of lung metastasis was significantly lower in the treatment group at day 40 in a higher metastatic potential model (MHCC97H: 2/8 vs 7/8, $p = 0.041$; MHCC97L: 0/8 vs 4/8, $p = 0.077$). Suppression of hepatic stellate cell activation was mainly found in the combination treatment groups. The tumor invasiveness including venous invasion/tumor thrombus was mainly presented in the control groups. The tumor proliferation was significantly suppressed by both single and combination treatments. The RNA and protein expression of Rho, ROCK and VEGF was down regulated in the combination treatment group.
Conclusion
Low dose rapamycin and FTY720 combination therapy significantly inhibited liver tumor growth and lung metastases.

158 POSTER

Targeting bladder tumor cells in vivo and in the urine by a peptide identified using phage display

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Bladder cancer is one of the most common tumors of genitourinary tract. Selective delivery of drugs to tumor tissues is important for effective tumor therapy. Here we identified a peptide targeting bladder tumor cells using phage display. A phage library containing CX7C random peptides was screened for selective binding to cells from human bladder tumor xenograft. Selected phage clones were individually evaluated for binding to cultured bladder tumor cells and for binding to cells from fresh human tumor tissues over cells from normal bladder tissues. The displayed peptide of the most promising clone was synthesized and named as Bld-1. Fluorescein-conjugated Bld-1 peptide showed selective binding to frozen sections of human bladder tumor tissues, while little binding to normal bladder tissues was observed. In vivo tumor targeting was examined in a carcinogen-induced rat tumor model. When the fluorescent peptide was instillated into the bladder lumen, it selectively bound to tumor epithelium, while little binding was observed in normal bladder epithelium. Moreover, when the fluorescent peptide were intravenously injected into the tail vein, it homed to the bladder tumor, while not detected in normal bladder and control organs such as lung. The peptide distributed in the tumor tissues in colocalization with cykokeratin, an epithelial cell marker. Next, we examined whether the peptide can detect tumor cells in the urine. The fluorescent Bld-1 peptide bound to urinary cells collected from tumor patients, while little binding was observed in urinary cells from healthy individuals. These results indicate that the Bld-1 peptide could be useful for targeting bladder tumor cells in vivo and in the urine.

159 POSTER

Limited penetration of paclitaxel and doxorubicin in multicellular layers of human cancer cells

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The multicellular layer (MCL) is a 3D in vitro model which resembles solid tumor conditions in vivo. Many studies reported different pharmacodynamics of chemotherapeutic agents between monolayers and 3D culture models. Paclitaxel (PTX) and doxorubicin (DOX) are widely used in the treatment of human solid tumors. The cells grown as MCL showed decreased sensitivity to both PTX and DOX compared with monolayers indicating the relative resistance of the cells in 3D condition, which can be attributed to the limited drug penetration. In the present work we used MCL of DLD-1 human colorectal adeno-carcinoma cells (Pgp + cells) to study the penetration of PTX and DOX into multilayers of human cancer cells. PTX (50 μM) showed slow penetration over 72 hr. Lower concentration of PTX (1 μM) failed to obtain a significant accumulation up to 48 hr exposure. Under drug exposure of 24 μM·hr, the penetration/accumulation of PTX was greater with higher drug conc rather than longer exposure. Dox showed a relatively faster penetration compared to PTX, i.e. 100 μM DOX showed a full penetration within 1 hr. However, lower concentration of DOX (10 μM) also failed to show a significant accumulation after 4 hr exposure. Another P-gp substrate, Calcein-AM, showed moderate penetration through the MCL after 2 hr of exposure, and inhibition of P-gp activity (incubation at 4ºC) induced only partial enhancement of penetration. These data indicate that P-gp substrates, PTX, DOX, and calcein-AM, are not similar in their penetration through the MCL of Pgp + cancer cell layers. Ethidium homodimer-1 (EthD-1), a highly hydrophilic agent also failed to penetrate through the MCL after 2 hr similar to PTX. Overall, our data suggest that not only physicochemical property(hydrophilicity) and P-gp affinity but also other factors such as tissue binding may be important factors in MCL penetration. Extensive tissue binding of PTX and DOX may hinder the penetration within avascular regions of human solid tumors in vivo, which warrants further investigation.

160 POSTER

In vitro and in vivo evaluation of pegaspargase for the treatment of solid tumors and lymphomas

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Background: Pegaspargase (Oncaspar®) is a PEGylated version of *E. coli* L-asparaginase (ASNase), and is approved for use in patients with acute lymphoblastic leukemia. Previous work suggests that ASNase may be efficacious in solid tumors and lymphomas and that its activity may correlate with the cellular levels of asparagine synthetase (ASNS). We evaluated the *in vitro* and *in vivo* efficacy of pegaspargase in pancreatic, ovarian and lymphoma cells with varying expression of ASNS.
Methods: The *in vitro* cytotoxicity of pegaspargase was determined using a MTS assay and levels of ASNS were measured by a quantitative RT-PCR. Pharmacokinetics (PK) and pharmacodynamics (PD) of pegaspargase were studied in rats and therapeutic efficacy was evaluated in xenograft models of pancreatic cancer.
Results: *In vitro*, pegaspargase had potent cytotoxicity against MiaPaCa-2, PANC-1 and Panc 10.05 pancreatic cells, and OV-90 and TOV-21G ovarian cells with IC_{50} values below 1.0 IU/mL. Similar cytotoxicity was observed against B- and T-lymphoma lines including Raji, Daudi, Molt-4 and Ramos. In a low ASNS-expressing model, MiaPaCa-2, treatment with a single dose of 12.5 IU/g pegaspargase resulted in 46% tumor growth inhibition (TGI). Further, although treatment with gemcitabine alone (80 mg/kg q3d × 4) or with low dose pegaspargase (0.8 IU/kg, single dose) alone was not significantly better than controls, treatment with the combination of the two resulted in improved efficacy compared to controls ($P < 0.05$) and a TGI of 48%. In contrast, in a high ASNS-expressing pancreatic model, ASPC-1, treatment with pegaspargase at various doses was ineffective. In PK/PD studies, the C_{max} and $AUC_{0-\infty}$ of pegaspargase increased and asparagine (ASN) levels decreased in a dose-proportional manner when pegaspargase was dosed via either intramuscular (IM) or intravenous (IV) routes. The elimination half-lives by IM or IV routes were comparable. ASN levels depleted rapidly following pegasparagase treatment and recovered with low dose but not with high dose treatment.
Conclusions: Pegaspargase had potent cytotoxicity against various pancreatic, ovarian and lymphoma cells and had significant antitumor efficacy in a xenograft of pancreatic cancer. The therapeutic efficacy of pegaspargase correlates with cellular ASNS which could serve as a biomarker in clinic. Pegaspargase either as a single agent or in combination with gemcitabine should be evaluated clinically for the treatment of solid tumors and lymphomas.