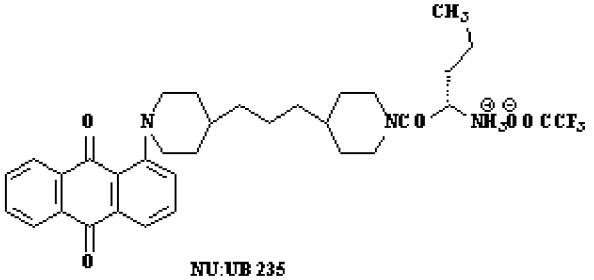
long infusion times for patients. Strong motivation thus exists for the design of new anti-topo I agents that stabilise cleavable complexes without the drawback of hydrolytic lability problems.
NU:UB 235, a spacer-linked, conformationally restricted anthracenedione conjugate of the unnatural amino acid norvaline, is representative of a new rationally designed series of topoisomerase I inhibitors.
NU:UB 235 has broad spectrum cytotoxic potency against a panel of human and animal tumour cell lines, with mean GI_{50} value ~1 μM in the NCI 60 cell line panel and transplantable, refractory MAC15A adenocarcinoma of the colon.
Enzyme-mediated relaxation of supercoiled pBR322 plasmid by either topo I or the α or β-isoforms of human topo II at concentrations up to 100 μM was not observed with NU:UB 235, compared to standard agents. However, the norvaline conjugate stabilised cleavable complex formation *in vitro* and reproducibly gave mean increases of 170% in nicked plasmid formation compared to 80% with camptothecin at equimolar concentrations (50 μM). This approximately 2-fold increase over camptothecin was observed also with the alternative ϕ X174RF plasmid.



NU:UB 235

Additionally, NU:UB 235 stabilised cleavable complex formation with topo I in intact HL60 leukaemic cells [following 45 minute exposure at 200 μM]. At equimolar concentrations, immunoband band depletion, by NU:UB 235, of the topo I Western blot signal in HL60 extracts exceeded (by 5-fold) that produced by camptothecin. The level of DNA damage induced by topo I poisoning is consistent with the non-intercalating, DNA groove-binding properties (Q_{50} value 0.9 μM for Hoechst 33258 displacement) of NU:UB 235, measured by fluorescence quenching.
Continuing pre-clinical development is warranted, given the demonstration that structurally stable NU:UB 235 is an effective non-camptothecin topo I poison.

514 POSTER

Pharmacokinetic study of the distribution, metabolism and excretion of non-radiolabeled DX 8951f following repeated intravenous administration to patients with solid tumors

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Background: The novel camptothecin derivative, DX 8951f (exetecan; D), is a potent topoisomerase I inhibitor with activity in the treatment of solid tumors. A pharmacokinetic (PK) study was designed to characterize the distribution, metabolism and excretion of *non-radiolabeled D* administered intravenously over 30-minutes daily for 5-days every 3 weeks by determining: the plasma PK, urinary and fecal excretion of D, D lactone and the two known metabolites of D, UM1 and UM2. Concentrations of D in saliva and *in vivo* plasma protein binding were also determined.
Methods: Patients were stratified into minimally (MP) and heavily pretreated (HP) groups based on prior treatment. Starting doses of D for MP and HP were 0.5 mg/m^2/day and 0.3 mg/m^2/day, respectively. Since D is predominantly metabolized by CYP3A4 and CYP1A2 enzymes, the enzyme activity was assessed in each patient pre-treatment by erythromycin breath test and caffeine urinary test, respectively. Limited blood samples were collected post-treatment, from days 1–6. Urine was collected continuously for 10-days and feces for 10–14 days during course 1. Saliva was collected at baseline and concomitantly with plasma samples, on day 1. Patients were hospitalized for up to 14 days of course 1. A recovery of 70% of D and its metabolites in all bodily secretions was defined as the apriori mass balance target.
Results: Twelve patients; 5♂:7♀; median age 57 (range 20–66); ECOG PS 0–1 (n=10), 2 (n=2) received 29 courses (median 2.5; range 1–5) of D. Tumor histology were pancreas (2), hepatocellular (2), renal/adrenal (2), gall bladder (1), esophageal (1) and others (4). Grade 4 neutropenia and thrombocytopenia were the predominant hematologic toxicities. Non-hematologic toxicities included nausea, emesis, fatigue and abdominal cramps. Overall, the total drug recovery ranged from 21.4 to 57.7% of the total administered dose of D. About 10% of D was eliminated unchanged in urine. UM1 and UM2 metabolite appeared to account for up to 25% and 15% of the total D administered, respectively. The PK parameters Clearance, half-life and Vss estimated noncompartmentally for D were within 2 standard errors of the values previously reported. The plasma exposure to UM-1 and UM-2 was approximately 4.3% and 1.3% of the parent compound.
Conclusions: Up to 57.7% of the administered dose of D is recovered in urine, feces and emesis fluid. Because less than 70% of the administered dose is accounted for by the parent drug and the two major metabolites, additional as yet unrecognized excreted metabolites may be present.

Topoisomerase II inhibitors

515 POSTER

Induction of unique structural changes in guanine-rich DNA regions by the triazoloacridone C-1305, a topoisomerase II inhibitor with potent activity toward solid tumors

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C-1305 is a triazoloacridone with excellent activity in lung and colon cancer models. We have recently reported that C-1305 is a topoisomerase II poison which is able to induce topoisomerase II-mediated cleavable complexes *in vitro* as well as in living cells. An unusual feature of C-1305 is the induction of low levels of very toxic cleavable complexes in tumor cells. To explore the molecular mechanisms underlying this phenomenon, we have investigated the *in vitro* sequence specificity of DNA binding for C-1305 and other triazoloacridones in comparison with classical topoisomerase II inhibitors and other DNA binders.
A 3′-end labelled 176 bp DNA fragment from pBluescript plasmid was used for studies on drug-DNA interactions. To determine the sequence specificity of DNA binding of triazoloacridone derivatives, DNA footprinting, chemical probing of DNA with DEPC and osmium teraoxide were used. Surface plasmon resonance (SPR) experiments were undertaken to determine the DNA binding affinity of C-1305 and C-1533 compounds. For determination of structure-activity relationship, thermal denaturation and chemical probing with DEPC was performed for a series of triazoloacridone.
Compound C-1305 shows almost 10 times higher preference for GC rather than AT DNA sequences as revealed by SPR ($K=3.0\times10^5$ M^{-1} for GC-rich DNA compared to 4.9×10^4 M^{-1} for AT-rich DNA). Chemical probing with DEPC showed that C-1305 induced structural perturbations in DNA regions with at least three consecutive guanine residues. This effect was detectable already at nanomolar concentrations of C-1305 and was highly specific for the C-1305 derivative, since none of the 22 other DNA-interacting drugs tested were able to induce similar structural changes in DNA. Structure-activity relationship studies with a series of triazoloacridone derivatives representing different chemical structures indicated that a hydroxyl group in the 8 position of the triazoloacridone ring as well as an aminoalkyl side chain containing three methylene groups are crucial for the unusual interaction of C-1305 with guanine-rich DNA regions.
We here show that the topoisomerase II inhibitor triazoloacridone C-1305 binds strongly to DNA at guanine-rich regions resulting in unique conformational alterations. Our results suggest that C-1305 might specifically influence the expression of genes that are regulated by guanine-rich elements in the promoter regions.

516 POSTER

Disruption of PKCzeta elicits hypersensitivity to submicromolar amounts of etoposide independently of the non-homologous end-joining pathway

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Background: To investigate the mechanisms by which wortmannin, a phosphatidylinositol 3-kinase (PI3K) inhibitor known to inactivate ATM