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Pharmacokinetics of intrathecal partaject busulfan in a phase I trial for patients with neoplastic meningitis

W. Petros[1], H. Friedman[2], K. Besas[2], M. Glantz[3], H. Sands[4], S. Tourt-Uhlig[2], E. Stewart[2], S. Gururangan[2], A. Friedman[2], J. Quinn[2]. *[1]West Virginia University Health Science Center, Mary Babb Randolph Cancer Center, Morgantown; [2]Duke University Medical Center, Duke Comprehensive Cancer Center, Durham; [3]Neurological Research Center, Inc., Bennington; [4]Supergen, Dublin, CA, USA*

We recently reported the results of a phase I study of intrathecally-administered busulfan in adult patients with neoplastic meningitis using a microcrystalline formulation, Partaject(TM) busulfan (Proc Am Soc Clin Oncol 2002;318a). Anti-cancer efficacy was noted, with minimal toxicity encountered to date. We now describe the previously unreported cerebrospinal fluid (CSF) and plasma pharmacokinetics of busulfan in patients treated on the phase I trial. The investigational formulation was administered via injection by the intralumbar or intraventricular route. Adult patients with neoplastic meningitis were enrolled using a limited escalation dose schedule design. Timed, serial plasma and CSF samples (seven of each type per patient) were obtained up to 5 hours following the first injection. Busulfan CSF and plasma concentrations were determined using a validated gas chromatographic assay. Pharmacokinetic parameter estimates were generated via a standard, two-stage, non-compartmental approach. Pharmacokinetic data are available in 23 patients treated on the following dose levels: 2.5, 5, 7.5, 10, 13, 17, 21.25 and 27 mg. The mean values for busulfan CSF clearance and half-life were 2.0 mL/minute and 64 minutes, respectively, each varying over a 10 fold range. No evidence for dose-dependent changes in clearance or half-life were observed. Both the AUC and Cmax values achieved in patients at the 21.25 mg dose level (26 mg/mL*min and 269 mcg/mL, respectively) were approximately 100 fold higher than the typical busulfan plasma AUC and Cmax observed when oral busulfan (1 mg/kg q6h) is used for ablative therapy in stem cell transplantation regimens. Some plasma samples had minimal busulfan concentrations, just above the limit analytic quantitation.

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Cellular and animal pharmacology of isophosphoramide mustard (IPM)

G. Bastian[1], N. Germann[1], S. Urien[1], A.H. Rodgers[2], L.R. Morgan[2], R.F. Struck[4], W.M. Waud[3], D.G. Serota[3], M. Ratterree[5]. *[1]Centre Rene Huguenin, Pharmacology, Saint Cloud, France; [2]DEKK-TEC Inc., New Orleans, USA; [3]MPI Research, Mattawan, USA; [4]Southern Research, Birmingham, USA; [5]Tulane Primate Center, Covington, USA*

Isophosphoramide mustard (IPM) is the active alkylating metabolite of ifosfamide. IPM is being prepared for a Phase I clinical trial - sponsored by DEKK-TEC with NCI support.In the present studies, IPM was administered by infusion once daily $\times$ 3 days to 16 adult beagle dogs with dose escalation between 1 and 100 mg/kg; by one bolus to 2 monkeys (5mg/kg) and to 10 mice (100mg/kg). Blood was drawn after IPM administrations during 24h for PK studies. The samples were extracted, chemically derivatized and assayed by GC-MS with good reproducibility (85%) and a quantification limit of 100 ng/ml. For dogs receiving 5 mg/kg (median) - T1/2 was in the range of 25 min. Plasma clearance was constant between 5 and 10 mg/kg (1.40 L/h/kg) and increased at 100 mg/kg (5.7 L/h/kg). Sparse data for 3 mg/kg prevented interpretations. At 5 mg/kg no IPM was detectable 1.5h after injection; at 10 mg/kg after 2h and at 100 mg/kg after 4h. MTD in dogs was 5 mg/kg. Toxicity noted was death at doses $>$10 mg/kg (renal toxicity) and at 5 mg/kg or less, transient liver and renal, bone marrow, and gastrointestinal dysfunctions although no IPM was detected in plasma. For mice, plasma concentrations decreased in $<$1 hour, with a clearance of 8.44 L/h/kg; T1/2 was 3.42 min (two compartment model). For monkeys mean T1/2a value was 4.86 min and T1/2b was 256 min. Median clearance was 1.65 L/h/kg and no IPM was detected 4h after dosing. No major toxicities were observed at this level. No potential IPM metabolites could be detected. In human blank plasma, 90% of IPM was bound to proteins within 5min of incubation. Human pharmacokinetic parameters were predicted from allometric analysis using the three species. Data predicted an acceptable starting dose of 30 mg/m^2 with a clearance of 40.5 L/h, a T1/2a of 10 min and a T1/2b of 1h45 min for a 70 kg patient. This is the proposed starting dose. Cellular studies were conducted with A549 cells (human lung cancer) *in vitro*. Various times of IPM incubation (at various doses) were tested; 10% of IPM was still detected after 24 h incubation at doses of 100, 250 and 500 mg/L. IPM uptake and efflux were studied, IPM was essentially in the nuclear compartment (90%). Effects of IPM on cell cycle was investigated by flow cytometry: S-phase block increased by 30%, as compared to control. The latter studies were attempts to document comparable tissue/IPM interactions that could occur under *in vivo* exposures. Supported by NCI/SBIR grant 44 CA 83552.

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Pharmacokinetics of D709119 (DRH-417), a DNA minor groove-binding pyrrolobenzodiazepine monomer with a novel mechanism of action

G.P. Wilkinson[1], T.C. Jenkins[2], D.E. Thurston[3], P.W. Howard[3], J.A. Double[1], M.C. Bibby[1], P.M. Loadman[1]. *[1]University of Bradford, Cancer Research UK Cancer Research Unit, Bradford, United Kingdom; [2]University of Bradford, Yorkshire Cancer Research Laboratory of Drug Design, Bradford, United Kingdom; [3]University of London, Cancer Research UK Gene Targeted Drug Design Group, London, United Kingdom*

D709119 is a DNA minor groove-binding pyrrolobenzodiazepine derivative that shows significant *in vitro* cytotoxicity towards a select number of cell lines in the 60-cell line NCI screen. This activity translates into human tumour xenograft models where *in vivo* antitumour activity has been demonstrated for a number of models including melanoma, renal and ovarian. On the basis of this *in vivo* activity, together with the novel mechanism of action of D709119 as suggested by findings from an NCI COMPARE analysis, the compound is now in pre-clinical development with the EORTC. The aim of this study was to develop a highly selective LC/MS analytical method in order to investigate the pre-clinical pharmacokinetics of D709119 in mice. A further goal was to measure tumour concentrations of the drug, and also brain concentrations in order to assess the degree of CNS penetration. An LC/MS-based method has been developed using an acetonitrile/ammonium formate mobile phase with a Hypersil phenyl reversed-phase HPLC column. Selective detection is achieved using electrospray MS analysis with a SIR of m/z 368.4 to detect the parent ion, the limit of detection is 50 nM. All *in vivo* studies have been approved by the UK Home Office. Pharmacokinetic studies have been carried out in NMRI mice bearing either MAC 29 or MAC15A murine colon tumours. Although an extraction efficiency of *50 % was achieved from plasma and brain homogenates, only $\sim$15 % extraction was achieved from spiked tumour homogenate suggesting extensive binding to tumour proteins. After i.p. administration at the MTD of 0.5 mg/kg, D709119 was easily detectible in mouse plasma up to 4 h post-dose, with peak concentrations of 171 nM after 30 min and a t1/2 of 2.3 h. The AUC was calculated to be 0.54 uM h. D709119 levels were below the limit of detection in both brain and tumour homogenates. An IC_{50} value for D709119 was determined experimentally in the human ovarian adenocarcinoma cell line SK-OV-3 to be 2.75 nM. These studies suggest that D709119 is bioavailable following i.p. injection at plasma concentrations well in excess of those necessary to achieve *in vitro* cytotoxicity. Further studies are now underway to probe the extensive binding of D709119 in tumour tissue.

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Intracellular and *in vivo* distribution of the pyrrolobenzodiazepine dimer SJG-136, a novel sequence-selective DNA minor groove cross-linking agent

G.P. Wilkinson[1], P.M. Loadman[1], J.P. Taylor[1], T.C. Jenkins[2], J.A. Double[1], S.J. Gregson[3], P.W. Howard[3], D.E. Thurston[3]. *[1]University of Bradford, Cancer Research UK Cancer Research Unit, Bradford, United Kingdom; [2]University of Bradford, Yorkshire Cancer Research Laboratory of Drug Design, Bradford, United Kingdom; [3]University of London, Cancer Research UK Gene Targeted Drug Design Resear, London, United Kingdom*

The pyrrolobenzodiazepine dimer SJG-136 is a novel sequence-selective DNA minor groove cross-linking agent with potent DNA stabilising activity and remarkable *in vitro* cytotoxicity (e.g. IC_{50} value of 23 pM in A2780 cells). The aim of this study was to characterise the *in vitro* cellular distribution of the agent and to establish its *in vivo* preclinical pharmacokinetic properties. SJG-136 is highly fluorescent and this enables its *in vitro* visualisation. Cell lines selected from the NCI's 60-cell line panel were treated with SJG-136 and fluorescence microscopy was used to monitor cellular uptake. It was demonstrated to produce a high level of nuclear-specific staining in HCT-116, UACC62, SK-MEL-2, SK-OV-3 and M14 cell lines, indicating both cellular penetration of the drug and localisation by covalent fixation within the nucleus, the proposed site of drug action. A sensitive and reproducible HPLC-based analytical method has been developed for SJG-136 that will be used to obtain pharmacokinetic data in forthcoming clinical trials. Using