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ANTITUMOUR, PHARMACOKINETICS AND DISTRIBUTION STUDIES OF DIFLOMOTECAN (BN80915) ADMINISTERED INTRAVENOUSLY TO *NUDE* RATS XENOGRAFTED WITH HUMAN NSC NCI-H460 LUNG TUMOUR CELLS

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EXPERIMENTAL METHODOLOGY

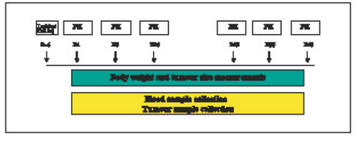
- Test substance: Diflomotecan (BN80915) prepared in 2% DMA, 2% Tween 20 and 96% - 24mM KH2PO4 pH 5.0
- Tumour cell line: Human NCI-H460 non small cell lung cancer
- Animals: *Rowett Nude* rats (Harlan SD Inc., Indianapolis)
- Drug administration: 10 min-intravenous (IV) infusion of diflomotecan to *Nude* rats *via* a catheter coupled to a pump-syringe.

PHARMACOKINETIC AND BIODISTRIBUTION OF DIFLOMOTECAN IN TUMOUR BEARING NUDE RATS AFTER A SINGLE IV INFUSION

- Subcutaneous inoculation of 10^6 NCI-H460 cells in 30 healthy *Nude* rats and randomisation of rats when the mean (± SD) tumour volume reached 459.1 ± 102.7 mm^3
- Anesthesia of rats with isoflurane
- 10 min single IV infusion of diflomotecan with an injection volume adjusted to the mean rat body weight (120 µl/min),
- Rat sacrifice by total blood sampling collection *via* the abdominal aorta at 5, 11, 20, 40, 70, 190, 310, 550, 730 and 1450 min after initiation of infusion (3 rats/sampling time),
- Collection of whole blood, plasma, tumour, liver, kidneys, lungs, heart, brain and intestine samples,
- Determination of diflomotecan concentration in plasma, tumour and tissues by HPLC/MS-MS validated methods using ^{13}C-BN80915 as internal standard,
- PK parameters were determined as area under the curve (AUC), plasma clearance (CL), terminal disposition half-life ($t_{1/2}$) and volume of distribution (Vd_{ss}) by non-compartmental analyses.

PHARMACOKINETIC, TOXICOLOGICAL AND ANTITUMOUR PROFILES OF DIFLOMOTECAN AFTER REPEATED IV ADMINISTRATIONS IN TUMOUR BEARING NUDE RATS

- Subcutaneous inoculation of $8x10^6$ human NCI-H460 NSCLC cells in 184 healthy *Nude* rats
- Rat randomisation when the mean (±SD) tumour volume reached 497 ± 137 mm^3,
- Treatment of rats done under anesthesia with ketamine / xylazine
- Repeated 10 min IV diflomotecan infusions at D1, D8, D15, D29, D36 and D43,
- Treatment schedule:



Pharmacokinetic analysis

- **Diflomotecan plasma pharmacokinetics at D1, D8, D15, D29, D36 and D43**
- **Pharmacokinetic analysis using a population approach (Nonmem)**
- **Tumour/plasma concentration ratio under steady state conditions**
  - Diflomotecan IV *bolus* injection at 0.5 mg/kg followed by a continuous IV infusion at 0.65 mg/kg over a 90 min period,
  - Rat sacrifice, blood and tumour samples collection at the end of infusion (90 min).

Toxicological and antitumour profiles

- **Monitoring of rat body weight and survival** *(Workman P. et al. 1998)*
  Every three days between D0 and D43
- **Rat hematological follow-up**
  Three times a week : white blood cells (WBC)/red blood cells (RBC)/platelets (PLT)
- **Monitoring of tumour volumes**
  Every three days between D0-D43
- **Monitoring of DNA Topoisomerase I cleavable complexes (DTCC) in tumour (Topogen kit)**

Introduction

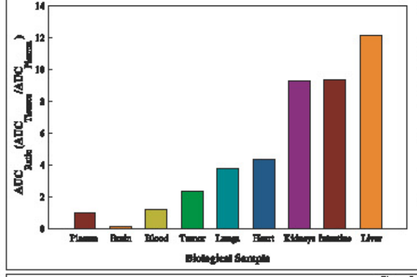
- Homocamptothecins (HCPTs) are novel anticancer drugs issued from research at Beaufour Ipsen,
- HCPTs inhibit topoisomerase I (Topo I) : in contrast with camptothecins they possess a 7-membered-beta-hydroxylactone ring that displays reduced electrophilicity leading to a considerable stabilization of the active, closed lactone form of the drug. As a consequence, the ring opening of HCPTs is also irreversible (Lavergne et al, 1997; Lesueur-Ginot et al, 1999),
- Diflomotecan (BN80915) is a difluorinated E-ring-HCPT derivative which has shown a specific Topo I inhibition coupled to a marked antitumor activity in various experimental human tumour models xenografted in Nude mice,
- Substantial safety and pharmacokinetic information has been gathered from phase I trials with diflomotecan administered intravenously (IV) and orally :
  - Diflomotecan induces predictable and manageable hematological toxicity
  - The development of diflomotecan as an oral Topo I inhibitor is supported by its superior oral bioavailability value coupled to a favourable safety profile after oral administration
- The objective of this study was to explore the PK/PD relationship in a model of Nude rat treated IV with diflomotecan

PHARMACOKINETIC AND BIODISTRIBUTION OF DIFLOMOTECAN IN TUMOR BEARING NUDE RATS AFTER A SINGLE IV INFUSION

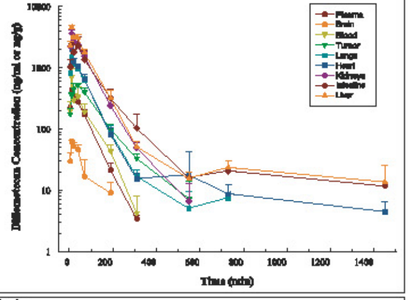
	C_{max} (ng/ml or ng/g)	T_{max} (h)	AUC (ng.h/ml or/and g)	$T_{1/2}$ (h)	CL ($L.kg^{-1}.h^{-1}$)	Vd_{ss} ($L.kg^{-1}$)
Plasma	443	0.18	548	0.82	1.82	2.16
Tumour	523	0.66	1330	1.42	-	-

Diflomotecan levels have been found higher in tumour rather than in plasma samples

VALUES OF AUC RATIOS (TISSUES AND TUMOUR *VERSUS* PLASMA) CALCULATED IN BIOLOGICAL SAMPLES FROM TUMOUR BEARING *NUDE* RATS TREATED WITH A SINGLE IV INFUSION OF DIFLOMOTECAN



COMPARATIVE DIFLOMOTECAN CONCENTRATIONS IN PLASMA, TUMOUR AND TISSUE SAMPLES FOLLOWING A SINGLE IV DOSE OF DIFLOMOTECAN



Conclusion

- The NCI-H460 tumour bearing Nude rat model was used to determine simultaneously the PK parameters, tissue distribution and antitumour activity of diflomotecan, after single and repeated IV infusions,
- After a single IV infusion, diflomotecan concentrations in tumour were higher than in plasma (AUC tumour/plasma ratio at 2.4),
- Similar diflomotecan PK profiles were obtained following repeated IV infusions, although lower plasma clearance values appear after repeated dosing,
- Under steady state conditions, the tumour/plasma concentrations ratios were 1.6 – 0.7
- No major toxicity of diflomotecan was observed in human NSCLung NCI-H460 cancer model in *Nude* rats after repeated IV infusions,
- A significant diflomotecan antitumour activity was induced in the NSCL NCI-H460 tumour xenografted in Nude rats,
- The antitumour activity of diflomotecan was reflected in significant tumour growth inhibition and a parallel increase of DNA topoisomerase I cleavable complexes,
- The *Nude* rat model used in this study appears suitable to relate the diflomotecan exposure (AUC) to the induced hematological toxicity and anti-tumour activity
- The analysis of the PK/PD relationship is of value in predicting the toxicity and the efficacy for a given dose regimen, key parameters for the clinical development of a new anti-cancer agent