MPC-6827, a Small Molecule Inhibitor of Microtubule Formation with High Brain Penetration: Absorption, Distribution, Metabolism, Excretion, and Clinical Considerations

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ABSTRACT

MPC-6827 is a new drug candidate that displays proapoptotic activity, with potency at low nanomolar concentrations in multiple cancer types including pancreatic, prostate, breast, colorectal, non-small cell lung, small cell lung, melanoma, ovarian and leukemia (Abstracts 5361 and LB252, 95th Annual Meeting of the AACR, 2004). The ADME (Absorption, Distribution, Metabolism and Excretion) properties of MPC-6827 were studied to facilitate the design and implementation of clinical studies. MPC-6827 was incubated *in vitro* in suspensions of rat and human liver microsomes and S9 fractions. Samples were analyzed by mass spectrometry to identify primary and secondary metabolites and to predict metabolic fate. Urine from rats treated with MPC-6827 was examined by mass spectrometry for confirmation. Rat and human plasma fortified with MPC-6827 was dialyzed to equilibrium to estimate plasma protein binding. *In vitro* activity of MPC-6827 against cell lines overexpressing the multidrug resistance pumps MDR-1, MRP-1 or BCRP was evaluated to estimate the potential for efflux. *In vivo* experiments in mice provided pharmacokinetic information on the tissue distribution of MPC-6827. MPC-6827 is highly protein bound: 98.3% in rat plasma and 98.2% in human plasma. The primary metabolic products of MPC-6827 are the *O*-demethyl metabolite and its glucuronide and sulfate conjugates, all found predominantly in urine. MPC-6827 distributes rapidly and extensively into the CNS, exhibiting 14-fold higher brain exposure relative to plasma and an elimination half-life similar to plasma. In addition, MPC-6827 is not a substrate for the main multidrug resistance pumps. These ADME properties suggest that MPC-6827 may be well suited for treatment of primary or metastatic tumors arising within the CNS; tumors that have progressed despite best standard treatment; or tumors that are no longer sensitive due to multidrug efflux pumps. These targets are the subject of Phase 1 clinical studies.

EXPERIMENTAL

Metabolite Identification

Incubation of MPC-6827 in Liver Microsomes: MPC-6827 in DMSO was added (to a final concentration of 10 µM) to human or rat liver microsomal suspensions (0.5 mg/mL protein) in 100 mM potassium phosphate buffer (pH 7.4) at 37 ºC. The metabolic reaction was initiated by the addition of NADPH (1 mM) or buffer (no NADPH control). The enzymatic reactions were terminated 40 minutes after initiation by the addition of 2 mL ethyl acetate. After mixing and centrifugation, the organic layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 40 ºC. Samples were reconstituted in 200 µL of 1:1 acetonitrile:water and submitted for mass spectrometry analysis.

Incubation of MPC-6827 in S9 Fractions: MPC-6827 in DMSO was added (to a final concentration of 10 µM) to human or rat liver S9 suspensions (0.5 mg/mL protein) in 100 mM potassium phosphate buffer (pH 7.4) at 37 ºC. The metabolic reaction was initiated by the addition of NADPH (1 mM) and UDPGA (0.15 mM) or buffer (no NADPH, UDPGA control). The incubation reactions were terminated at 40 minutes after initiation by the addition of 2 mL ethyl acetate. After mixing, and centrifugation, the organic layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 40 ºC. Samples were reconstituted in 200 µL of 1:1 acetonitrile:water and submitted for mass spectrometry analysis.

Excretion of MPC-6827 and its Metabolites in the Rat: A female Sprague-Dawley rat received a single intravenous dose of 2 mg/kg MPC-6827 in D5W. One milliliter of a 1 mg/mL citric acid solution was added to the urine trap of a metabolic cage. Urine and feces were collected over 24 hours. Urine was submitted for mass spectrometric analysis with no further processing. Feces were homogenized in 2 volumes acetonitrile:water (w/v), centrifuged and submitted for mass spectrometry analysis.

Pharmacokinetics in Nu/+ Mice

Male animals were dosed with 2.5 mg/kg MPC-6827 as a single IV injection via the tail vein. Blood samples and whole brains were collected from five mice at each of the nine collection time points of 0.05, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours post dose. Plasma was collected from blood samples, and whole brain samples were homogenized in three volumes water. Both tissues were analyzed for concentrations of MPC-6827 by liquid chromatography-mass spectrometry (LC-MS/MS).

***In vitro* Rat and Human Plasma Protein Binding**

In vitro percent plasma protein binding of MPC-6827 in both rat and human plasma was determined by equilibrium dialysis followed by LC-MS/MS analysis.

DATA ANALYSIS

Metabolism

Phase I metabolites were identified by comparing chromatograms from control samples (minus NADPH) to samples incubated in the presence of NADPH. The elution times, molecular weights and fragmentation patterns of suspected metabolites were compared to synthetic standards for positive identification. Phase II metabolites were identified by molecular weight shifts in positively identified phase I metabolites consistent with glucuronidation and sulfation. The site of phase II metabolism was determined by comparing MS/MS/MS fragmentation patterns of the phase II conjugates with the MS/MS fragmentation patterns of the Phase I synthetic standards.

Pharmacokinetic Analysis

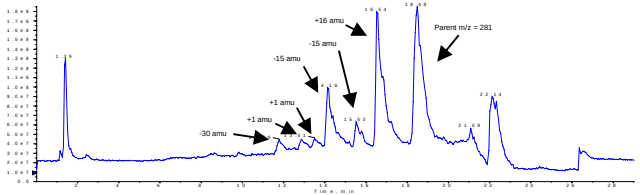
Pharmacokinetic parameters were estimated on median plasma and brain concentrations using non-compartmental analysis in WinNonlin (Pharsight Corp., Mountain View, CA). The areas under the concentration-time curve (AUC(0-inf)) were calculated using a linear/log trapezoidal method.

Protein Binding

The ratio of the MPC-6827 peak area to the peak area of the internal standard, was used in the formula below to calculate the percent plasma protein binding for each sample.

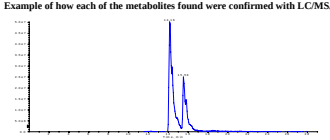
$$\% \text{ Bound} = \frac{[\textit{Plasma Compartment}] - [\textit{Buffer Compartment}] \times 100}{[\textit{Plasma Compartment}]}$$

Total Ion Chromatogram from Human Liver Microsome Incubation

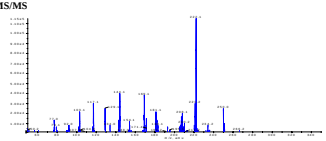


Comparison of *N*-demethylation product from Human Liver S9 Incubation and Synthetic Standard

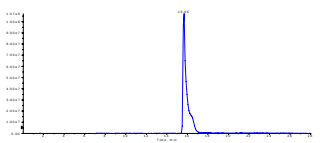
Example of how each of the metabolites found were confirmed with LC/MS/MS



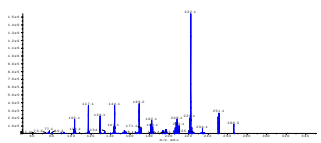
Extracted Ion Chromatogram (XIC), m/z = 266, from human liver S9 incubation showing two peaks with molecular weight consistent with de-methylation



Enhanced Product Ion (EPI) scan of 15.53 min peak from human liver S9 incubation



Extracted Ion Chromatogram (XIC), m/z = 266, of *N*-demethylation reference standard

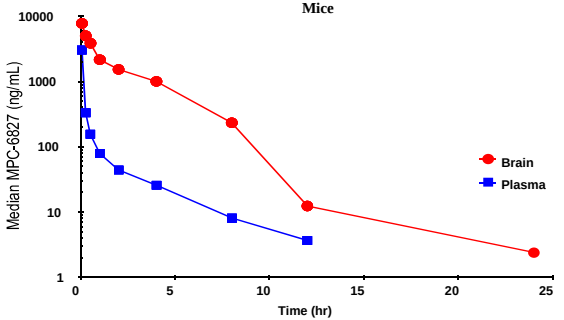


Enhanced Product Ion (EPI) scan of 15.66 min peak from *N*-demethylation reference standard

Summary of Metabolites Found

	Rat Microsomes	Rat S9	Rat Urine	Human Microsomes	Human S9
O-demethyl	X	X	X	X	X
N-demethyl	X	X		X	X
Di-demethylation	X	X		X	X
Methyl Hydroxylation				X	X
Unknown Hydroxylation	X		X		
Glucuronidation		*O*-demethyl site	*O*-demethyl site Unknown hydroxylation site		*O*-demethyl site
Sulfation		*O*-demethyl site	*O*-demethyl site		*O*-demethyl site

MPC-6827 PK Following a 2.5 mg/kg IV Bolus Dose in Male Nu/+ Mice



Tissue	$t_{1/2}$ (hr)	C_{max} (ng/mL)	$AUC_{(0\text{-}inf)}$ (hr*ng/mL)	CL (mL/hr/kg)
Plasma	2.75	3040	794	3150
Brain	2.08	7810	11095	N/A

Median *In vitro* Rat Plasma Protein Binding at a Final Concentration of 2.5 µg/mL (n=4)

Buffer Compartment (Analyte Peak Area/IS Peak Area)	Plasma Compartment (Analyte Peak Area/IS Peak Area)	% Plasma Protein Bound
4.24E-01	2.55E+01	98.3

Median *In vitro* Human Plasma Protein Binding at a Final Concentration of 2.5 µg/mL (n=3)

Buffer Compartment (Analyte Peak Area/IS Peak Area)	Plasma Compartment (Analyte Peak Area/IS Peak Area)	% Plasma Protein Bound
4.31E-01	2.41E+01	98.2

RESULTS & CONCLUSIONS

- The *O*-demethyl and *N*-demethyl metabolites of MPC-6827 were identified in incubation mixtures containing human liver microsomes. Their presence (and that of their glucuronide and sulfate conjugates) in rat urine was confirmed using mass spectrometry. Additional oxidative metabolites, identified as the di-demethyl and hydroxymethyl metabolites have also been found in urine. Each of these metabolites showed mass spectral and chromatographic characteristics identical to synthesized metabolite standards. Rat and human metabolites of MPC-6827 are qualitatively similar. Urinary metabolites in humans will be confirmed in ongoing clinical studies.
- MPC-6827 crosses the BBB and distributes rapidly into the CNS with exposure in the brain approximately 14 times higher than in plasma. These data suggest that it is possible to reach therapeutic drug concentrations in the CNS with minimal systemic exposure. This property suggests a unique opportunity to study antitumor activity in patients with primary brain tumors.
- *In vitro*, MPC-6827 is highly bound to plasma protein in rats (98.3%) and human plasma (98.2%). These data suggest that MPC-6827 may show high plasma protein binding in humans.