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ORIGINAL ARTICLE

Plasma and cerebrospinal fluid pharmacokinetics of MP470 in non-human primates

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Abstract
Purpose MP470 is a multi-targeted tyrosine kinase inhibitor with potent activity against mutant c-Kit, PDGFRα, Flt3, c-Met and c-Ret that is being evaluated as an anticancer agent. The plasma and cerebrospinal fluid (CSF) pharmacokinetics of MP470 were studied in a non-human primate model that is highly predictive of CSF penetration in humans.
Methods Oral MP470, 300 mg, was administered to four non-human primates. Serial samples of blood were collected from four animals and CSF samples from three animals for pharmacokinetic studies. Plasma and CSF concentrations were measured using an LC–MS/MS assay. Both model-independent and model-dependent methods were used to analyze the pharmacokinetic data.
Results Following a one-time oral dose of 300 mg, the MP470 plasma area under the curve (AUC) was 1,690 ± 821 nM h (mean ± SD). The half-life of MP470 in the plasma was 11.0 ± 3.4 h. There was no measurable MP470 in the CSF.
Conclusions Although CSF penetration is minimal, MP470 has demonstrated potent activity against cancer cell lines in vitro and in vivo, and further clinical investigation is warranted.

Keywords MP470 · CSF penetration · Pharmacokinetics · Tyrosine kinase inhibitor

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Introduction

MP470, 4-[1]benzofuro[3,2-*d*]pyrimidin-4-yl-*N*-(1,3-benzodioxol-5-ylmethyl)piperazine-1-carbothiamide hydrochloride, is a multi-targeted tyrosine kinase inhibitor that has demonstrated in vitro and in vivo activity against multiple validated cancer targets including mutant c-Kit, mutant PDGFRα, mutant Flt3, c-Met and c-Ret. MP470 has been shown to have potent activity against mutant c-Kit, suggesting a potential role in the treatment of tumors with activating or gain of function mutations in c-kit including gastrointestinal stromal tumors (GIST) [1–3], thyroid cancers [4], dysgerminomas [5], acute myeloid leukemia [6] and multiple pediatric tumors [7].

In addition to its activity as a single agent, MP470 has demonstrated synergistic activity with double-stranded DNA-damaging agents through the modulation of Rad51 expression, an essential protein in DNA repair mechanisms. High levels of Rad51 expression have been correlated to confer resistance to chemotherapeutic agents [8–10] and decreasing Rad51 increases cell sensitivity to ionizing radiation. Clinical data suggests that there is correlation between Rad51 expression and survival in patients with non-small-cell lung cancer (NSCLC) [8].

Preclinical animal experiments suggest that MP470 has a wide therapeutic index with little to no toxicity in the expected therapeutic dose range. When MP470 was used in combination with ionizing radiation in the treatment of glioblastoma multiforme cell lines, there was an over two-fold increase in cell death when compared with either treatment alone.

In order to assess the CNS penetration of MP470, we evaluated the plasma and CSF pharmacokinetics after oral administration using a non-human primate model that has been highly predictive of anticancer distribution in humans.

Materials and methods

Drug

MP470 hydrochloride capsules (100 mg) were supplied by SuperGen, Inc, Dublin California. The capsules were opened and the contents were mixed with yogurt or applesauce for oral administration.

Animals

The study was approved by the Baylor College of Medicine Institutional Animal Care and Use Committee. Four healthy adult male rhesus monkeys (*Macaca mulatta*) weighing between 11.1 and 16.3 kg were used in these experiments. The animals were fed Lab Diet 5045 twice daily and were group housed in accordance with the Guide for the Care and Use of Laboratory Animals [11]. Animals were observed daily for at least 4 weeks after MP470 dosing for any evidence of clinical toxicity. Clinical laboratory studies including complete blood counts, electrolytes, liver and renal function tests were obtained prior to MP470 administration and weekly thereafter for 4 weeks.

Experiments

Four animals received a single 300 mg oral dose of MP470 administered with food. Blood samples (3 ml) were obtained in 4 animals through a peripheral intravenous (IV), and CSF samples (0.3 ml) were obtained in 3 animals from an indwelling subcutaneously placed ommaya reservoir in the fourth ventricle. The reservoir was pumped three times prior to the collection of CSF to ensure adequate mixing with ventricular CSF. Samples were obtained prior to administration and at 1, 2, 4, 6, 8,10, 24, 30 and 48 h following the dose.

Sample analysis

Fifty ul samples, as well as standards and controls were aliquoted into each well of a 96-well plate, spiked with 5 µl of 1 µg/ml SSG-1097 (internal standard) and 5 ul of DMSO, and then mixed with 200 µl of acetonitrile to precipitate protein and extract MP470. Plates were shaken for 5 min at room temperature and centrifuged at 4,000 rpm at 10°C for 15 min. About 100 µl of each supernatant was transferred to a new conical bottom plate for LC–MS/MS analysis.

MP470 concentrations in plasma and CSF were measured by a LC–MS/MS system consisting of a Sciex 4000Q-Trap (Applied Biosystems, Foster City, CA) with a Shimadzu Prominent HPLC system (Shimadzu, Columbia, MD). The HPLC separation was achieved on a Phenomenex Luna C8, 50 × 2 mm, 3 µm column. The mobile phase consisted of a mixture of 0.05% formic acid in 5 mM ammonium acetate (solution A) and 0.1% formic acid in acetonitrile (solution B). The method used a linear gradient of 15–85% solution B from 0 to 2 min, followed by a 1.5 min hold, and a return from 85 to 15% solution B from 3.5 to 4 min with a 1 min hold. The total run time was 5 min and the flow rate was 300 µL/min. The data was analyzed using Analyst 1.4.2 software (Applied Biosystems), and the calibration curve regression was quadratic with a 1/× weighting. The concentration range for both plasma and CSF over which the calibration curve was acceptable was 1–1,000 ng/ml, with a correlation coefficient, $r > 0.990$. The lower limit of quantification (LLOQ) as defined by the lowest calibrator was 1 ng/ml. The intra-day precision was less than 4%.

Pharmacokinetics analysis

Standard pharmacokinetic parameters for MP470 in plasma, including C_{max}, terminal half-life, area under the concentration time curve (AUC), and volume of distribution, were estimated using model-independent (non-compartmental) methods. A linear least-square fit of the final four concentration–time points was used to estimate the terminal half-life. The AUC was calculated from the concentration time curves using the trapezoidal method and extrapolated to infinity using the terminal rate constant [12]. Apparent clearance was estimated as dose divided by AUC. Volume of distribution was calculated using the AUC and the area under the moment curve (AUCM). As a second analysis, compartmental modeling of the plasma concentration–time data was done with ADAPT-II using the maximum likelihood method [13].

Results

After oral administration of a 300 mg MP470 dose, the maximum plasma concentration was observed between 4 and 10 h after dosing and ranged from 32.6 to 240 nM. The

plasma $AUC_{(0-\infty)}$ was 1,690 ± 821 nM h and the fraction of the AUC that was extrapolated to infinity from the final time point at 24 h varied from 8 to 28%, with a median of 10%. Complete results of the non-compartmental pharmacokinetic analysis are shown in Table 1. There was no detectable MP470 in any of the CSF samples collected from the three animals.

The plasma concentration time data were best fit by a model that incorporated a time delay for absorption, first-order absorption kinetics, and first-order elimination kinetics (Fig. 1). The model fits for each of the four animals are shown in Fig. 2, and the model parameter estimates are shown in Table 2. Drug absorption was highly variable with an absorption half-life ranging from 0.36 to 6.4 h. The apparent volume of distribution (V/F) was also widely variable, ranging from 163 to 950 l/Kg.

The animals used in this study experienced some mild toxicity, which was likely attributable to the drug, including elevated temperature, apparent nausea, and a brief period of agitation and somnolence in one animal that resolved after additional anti-emetics. All animals returned to their pre-treatment state following therapy and had no significant adverse affects. All animals were pre-medicated with ondansetron prior to drug administration and received acetaminophen for elevated temperatures.

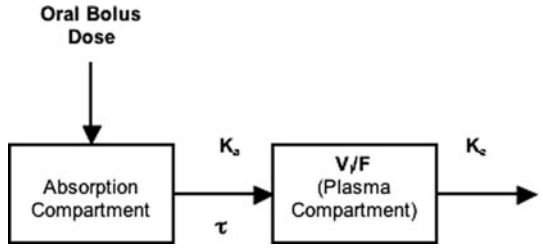

Fig. 1 Schematic of the PK model for MP-470 in non-human primates. The model uses first-order absorption with a variable time lag and linear elimination. *Legend* Ka-absorption rate constant, tau-absorption time lag, V1/F-apparent volume of distribution, and Ke-elimination rate constant

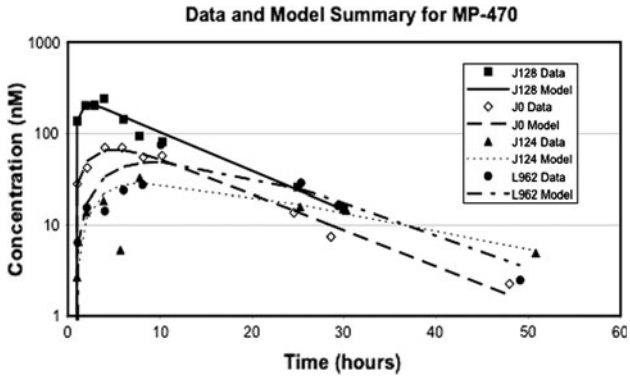

Fig. 2 Model predicted versus measured concentrations of MP470 in plasma of non-human primates. Measured concentrations are shown with symbols. Model estimates are indicated by *lines*

Discussion

MP470 pharmacokinetics after oral administration is characterized by first-order absorption and elimination. The time to peak and peak concentration were highly variable between animals with the T_{max} ranging from 4 to 10 h and a 9-fold difference in the C_{max}. There was no detectable MP470 in the CSF of any of the non-human primates studied. This is not totally unexpected since in vitro studies have demonstrated >98.8% protein binding in both rat and human plasma.

The wide inter-animal variation we observed in plasma C_{max} was also observed in studies of MP470 in healthy adults; in addition, administration with food increased Cmax threefold in adults [14]. The average C_{max} after a single oral dose of 300 mg in our study of 0.24 μM was less than the IC50 demonstrated in cancer cell lines in vitro. In two prostate cancer cell lines, the IC50 of MP470 was approximately 4 and 8 μM [15]. In small-cell lung cancer cell lines, for six of the seven cell lines the average IC50 was approximately 10 μM (range, 4.8–18.9 μM) [16]. In a study that evaluated single-dose pharmokinetics in healthy volunteers, after receiving an oral dose of 700 mg in both the fed and fasted state, C_{max} for male patients in the fed state was 221 ± 114 ng/ml (mean ± SD), (0.46 ± 0.24 μM) and 120 ± 77 ng/ml (0.25 ± 0.16 μM) for females [14]. To achieve concentrations closer to these published IC50 values, multiple day dosing and higher doses (leading to higher steady-state concentrations) will be required. The

Table 1 Non-compartmental pharmacokinetic parameters for MP470 after single oral dose in non-human primates

Animal	Dose (mg/kg)	C_{max} (nM)	AUC t-last (nM h)	AUC inf (nM h)	Vd/F (l/kg)	Clearance/F (ml/min/kg)	$t_{1/2}$ (hours)
Model independent							
J128	19.4	239	2,620	2,900	210	230	13.1
J0	27	70.0	1,190	1,290	611	719	9.7
J124	21.4	32.6	802	1,110	1,122	665	14.5
L962	18.4	74.6	1,300	1,460	494	434	6.90
Mean ± SD		104 ± 92.2	1480 ± 791	1,690 ± 821	609 ± 381	512 ± 225	11.0 ± 3.4

Table 2 Model-dependent pharmacokinetic variables

Animal	Ka* (h^{-1})	Tau (Min)	V/F (l/Kg)	Ke (h^{-1})
Model dependent				
J128	1.94	35.3	163	0.10
J0	0.41	13.9	544	0.09
J124	0.26	43.5	950	0.05
L962	0.11	39.2	303	0.11
Mean ± SD	0.68 ± 0.85	33 ± 13.2	490 ± 345	0.09 ± 0.03

Ka absorption rate constant, *Tau* absorption time lag

current Phase I study in small-cell lung cancer and neuroendocrine tumors has escalated to a M-470 dose of 500 mg every 8 h.

The toxicity observed in this single dose animal study of MP470 (nausea, somnolence, and elevated temperature) was mild and transient, similar to those observed in ongoing Phase I trials in adults. In one study in which MP-470 was used in combination with standard of care regimens in patients with small-cell lung cancer and neuroendocrine malignancies, adverse events which occurred in greater than 10% of patients included alopecia, diarrhea, nausea, neutropenia, thrombocytopenia, constipation, fatigue, peripheral sensory neuropathy, and oral candidiasis [16].

Based on in vitro experiments, MP470 has activity against multiple validated cancer targets, such as c-Kit, mutant PDGFRα, mutant Flt3, c-Met, and c-Ret [1, 2, 17–22]. Although we could not quantitate MP470 in the CSF, this finding may not eliminate its potential utility in the treatment of CNS malignancies if adequate systemic exposures can be achieved in man. In some tumors, disruption of the blood brain barrier can result in significant intra-tumoral exposure to drugs that do not cross the intact blood–brain barrier [23]. Also since MP470 may affect maintenance of tumor vasculature, there is a potential beyond the direct tumor effects of this agent. Furthermore, MP470 in combination with radiation results in increased apoptosis and decreased dsDNA repair in vitro, and increased survival in xenograft models [24]. Further preclinical investigation into orthotopic tumor models, including studies that combine MP470 with other active agents that have greater CNS penetration may inform the potential for further development of this agent for the treatment of CNS malignancies.

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