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ORIGINAL ARTICLE

# Plasma and cerebrospinal fluid pharmacokinetics of pemetrexed after intravenous administration in non-human primates

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**Abstract**
*Purpose* Pemetrexed, a multi-targeted antifolate that disrupts synthesis of both purines and pyrimidines, is approved for use in malignant pleural mesothelioma and non-small cell lung cancer. Pemetrexed is currently being evaluated for anti-tumor activity in a variety of solid and central nervous system tumors. We studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of pemetrexed in a non-human primate model that is highly predictive of human CSF penetration.
*Methods* Pemetrexed, 20 mg/kg (400 mg/m$^2$), was administered intravenously to four non-human primates. Serial blood samples were obtained from all animals and serial CSF samples were obtained from three animals. Plasma and CSF concentrations of pemetrexed were measured using LC/MS/MS and the resulting concentration versus time data were evaluated using model independent and dependent methods.
*Results* Pemetrexed disappearance from plasma was best described by a two compartment model with a mean distribution half-life of 13.8 ± 3.2 min and an elimination half-life of 70.0 ± 16.0 min. The volume of distribution of and the clearance from the central compartment were 0.066 ± 0.017 l/kg and 3.6 ± 0.6 ml/min/kg, respectively. Peak CSF concentrations occurred 40–71 min after the start of the infusion with an average of 0.26 ± 0.15 μM.
*Conclusion* The CSF penetration of pemetrexed was less than 2% (range 0.33–1.58%), suggesting that it should be used in conjunction with other CNS preventive strategies when used in the treatment of malignancies with a predilection for CNS or leptomeningeal metastases.

**Keywords** Pemetrexed · Antifol · Antimetabolite · CSF penetration · Pharmacokinetics

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## Introduction

Pemetrexed, (*N*-[4-(2-[2-amino-4, 7-dihydro-4-oxo-1H-pyrrolo(2,3-D) pyrimidin-5-yl] ethyl) benzoyl]-L-glutamic acid), is a multitargeted antifolate antimetabolite (Fig. 1). Pemetrexed has a mechanism of action similar to methotrexate, which is a widely used antineoplastic agent for hematologic malignancies and solid tumors. Pemetrexed disrupts enzymes involved in both purine and pyrimidine synthesis, including thymidylate synthase, dihydrofolate reductase, and glydinamide ribonucleotide formyl transferase [1]. Pemetrexed is polyglutamated and retained intracellularly in its active form. In addition, the polyglutamated form is at least 60 times more potent in inhibiting thymidylate syn-

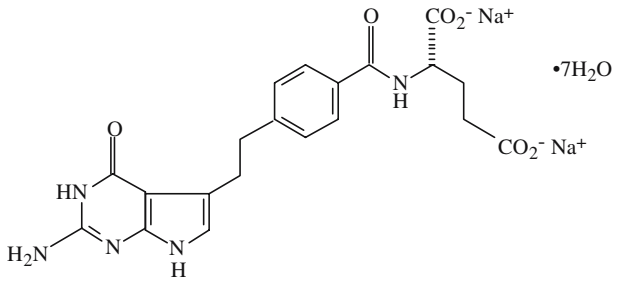


**Fig. 1** Chemical structure of pemetrexed

thase versus the parent form [1]. Pemetrexed is available commercially and is approved for use in malignant pleural mesothelioma as well as non-small cell lung cancer. It has also shown single agent activity in a variety of solid tumors including breast cancer [12]. Compared to methotrexate, pemetrexed has a different resistance pattern, indicating utility of pemetrexed in methotrexate resistant tumors [16].

The CSF penetration of pemetrexed has not previously been reported. In this study, the CSF and plasma pharmacokinetics of pemetrexed were examined in a non-human primate model that has previously been shown to be predictive of CSF penetration in humans [7].

## Materials and methods

### Drug

Pemetrexed was supplied by Eli Lilly and Company (Indianapolis, Indiana) in 500 mg vials containing 25 mg/ml of the disodium salt. The appropriate dose of drug was further diluted with NaCl to a final total volume of 50 ml (3.6–6.4 mg/ml) and administered over 10 min through an indwelling catheter in the jugular vein or a peripherally placed catheter in the saphenous vein.

### Animals

Four adult rhesus monkeys (*Macaca mulatta*) weighing 9–16 kg were used for this study. 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 [10]. Blood samples were obtained through either a central venous catheter (if not used for the drug infusion) or a peripheral catheter in the contralateral saphenous vein. CSF samples were obtained from a chronically indwelling subcutaneously placed Ommaya reservoir attached to a Pudenz catheter with its tip in the fourth ventricle. The reservoir was pumped three times before each CSF sample collection to ensure adequate mixing with ventricular CSF.

### Experiments

Pemetrexed was administered by a short (10 min) intravenous infusion at a dose of 20 mg/kg (400 mg/m$^2$) administered intravenously over 10 min. Serial blood samples were obtained prior to the infusion; at the end of the infusion; and at 15, 30 and 60 min, and 2, 4, 6, 8 and 24 h after the infusion. Serial CSF samples were obtained prior to the infusion; at the end of the infusion; and at 15, 30 and 60 min, and 2, 4, 6, 8 and 24 h after the infusion. Plasma was separated immediately by centrifugation at 4,400 rpm for 10 min at 5°C. Plasma and CSF samples were stored at –80°C until analysis.

### Sample analysis

Pemetrexed plasma concentrations were analyzed by a modification of the liquid chromatography mass spectroscopy/tandem mass spectroscopy (LC/MS/MS) method described by Chaudhary et al. [3]. The LC/MS/MS system consisted of a Shimadzu liquid chromatograph (Wood Dale, IL, USA) with two LC-10ADvp

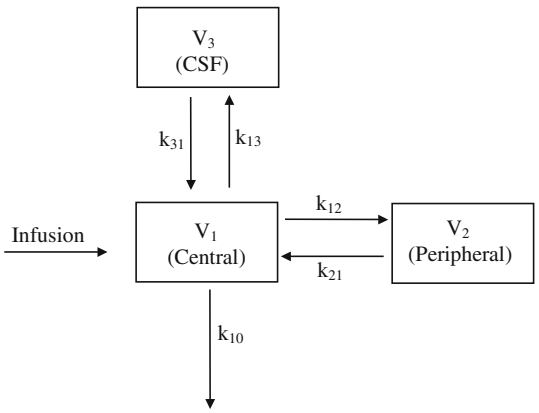


**Fig. 2** The pharmacokinetic model best describing the plasma and CSF disposition of pemetrexed consists of central and peripheral compartments with first order irreversible elimination from the central compartment and a third compartment for CSF. $V_1$ is the volume of distribution of the central compartment, $V_2$ the volume of distribution of the peripheral compartment, and $V_3$ the volume of the CSF compartment (fixed at 10 ml for this model). $K_0$ represents the drug infusion. The rate constant $k_{10}$ represents the elimination of pemetrexed from the central compartment, $k_{12}$ represents movement of pemetrexed from the central to the peripheral compartment, and $k_{21}$ represents the reverse. The rate constant $k_{13}$ represents movement of pemetrexed from the central compartment to the CSF, and $k_{31}$ represents the reverse

pumps (flow rate 0.200 ml/min), and a SIL-10ADvp autoinjector (injection volume 20 μl) coupled to a triple quadrupole Quattro Micro mass spectrometer (Waters Corporation, Milford, MA, USA) fitted with an electrospray ionization probe operating in the positive mode. Plasma samples (0.1 ml) containing pemetrexed and internal standard ($[^2H_4]$-pemetrexed) were precipitated with ice-cold methanol (0.2 ml). After vortex mixing for 15 s, samples were kept on ice for 15 min. Following centrifugation (14,000 rpm, 4 min) in a refrigerated centrifuge set at 4°C, the aqueous-methanol supernatant was filtered through a 0.45 μm Captiva plasma protein precipitation 96-well filter plate into a 96-deep well polypropylene collection plate using a 3 M Empore vacuum manifold under ~ 15–20 in./Hg vacuum. The sample was chromatographed under reverse phase conditions on a Genesis C18 analytical column (2.1 × 100 mm, 3 μm particle size) and a Brownlee NewGuard RP-18 precolumn (3.2 × 15 mm, 7 μm). The mobile phase was composed of water/acetonitrile (86/14) containing 0.2% formic acid and delivered at a flow rate of 0.2 ml/min. The injection volume was 25 μl. The compounds were detected and quantified by LC/MS/MS using electrospray ionization. The source temperature, desolvation temperature, cone gas flow and desolvation gas flow were 120, 350°C, 100 and 650 l/h, respectively. Pemetrexed detection was accomplished by MS/MS using the parent ion $m/z$ of 428.1 and the daughter ion $m/z$ of 281.1. The dwell time, cone voltage, and collision energy values were 0.2 s, 30 V, and 25 eV, respectively. $[^2H_4]$-pemetrexed was detected by MS/MS using parent ion signal of $m/z$ 432.1 and daughter ion signal of $m/z$ 285.1. The dwell time, cone voltage and collision energy values were 0.2 s, 30 V, and 20 eV, respectively. Mass spectra and chromatograms of pemetrexed and $[^2H_4]$-pemetrexed were processed using the Quanlynx routines in the MassLynx v3.5 software. Standard curves were generated using the peak area ratio of drug/internal standard samples containing known concentrations of drug. Standard curves were linear ($r^2 > 0.99$) over the ranges of 10–2,000 ng/ml (0.016–3.35 μM) and 1,000–200,000 ng/ml (1.67–335 μM). The coefficient of variation for standard values at the lower limit of quantitation (10 ng/ml, 0.016 μM) was 4.1%. There were two differences in sample preparation procedures for CSF and plasma. First, plasma samples were centrifuged prior to work-up to remove residual clotted protein. Second, standards for CSF analysis were prepared in human plasma ultrafiltrate (protein-free) which is preferred matrix that contains lipids and salts.

## Protein binding

Pemetrexed binding to proteins was measured in thawed human plasma, prefiltered human plasma, non-human primate plasma and phosphate buffered saline, pH 7.4. After 30-min incubation at 37°C, samples were added to the reservoir of a YM-30 Amicon Centrifree micropartition device (Millipore, Bedford, MA, USA). Ultrafiltration was accomplished by centrifugation (1,500$g$) in a fixed angle rotor for 30 min at 4°C after a 30 min incubation period at room temperature. Drug concentrations were measured in samples before (sample reservoir) and after (filtrate cup) centrifugation and the percentage of drug recovered and protein binding were calculated by the equations:

$$\text{Percentage recovered} = \left[\frac{\text{Filtrate cup concentration}}{\text{Sample reservoir concentration}}\right] \times 100\%$$

## Pharmacokinetic analysis

The area under the concentration versus time curve (AUC), total body clearance ($Cl_{TB}$), steady-state volume of distribution ($Vd_{ss}$), and mean residence time (MRT) were calculated using non-compartmental methods. The AUC was determined using the linear trapezoidal method and was extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant. $Cl_{TB}$ was derived by dividing the dose by the AUC. $Vd_{ss}$ was calculated using the area under the moment curve (AUMC), correcting for infusion time. The MRT was determined

**Table 1** Model independent plasma pharmacokinetic parameters of pemetrexed after a single intravenous infusion to non-human primates

| Animal | $C_{max}$ (μM) | AUC (μM h)$_{0 \to \infty}$ | $Cl_{TB}$ (ml/min/kg) | MRT (min) | $Vd_{ss}$ (l/kg) |
|---|---|---|---|---|---|
| J124 | 246 | 118 | 4.8 | 67 | 0.31 |
| J128 | 184 | 132 | 4.2 | 62 | 0.26 |
| L962 | 268 | 159 | 3.5 | 64 | 0.22 |
| L976 | 290 | 156 | 3.6 | 48 | 0.17 |
| Mean ± SD | 247 ± 40 | 141 ± 17 | 4.0 ± 0.5 | 60 ± 7.5 | 0.24 ± 0.05 |

**Table 2** Model dependent plasma pharmacokinetic parameters for pemetrexed after a single intravenous infusion to non-human primates

| Animal | $k_{10}$ ($min^{-1}$) | $k_{12}$ ($min^{-1}$) | $k_{21}$ ($min^{-1}$) | $k_{13}$ ($min^{-1}$) | $k_{31}$ ($min^{-1}$) | $V_1$ (l/kg) | $V_2$ (l/kg) | $Cl_{central}$ (ml/min/kg) | $t_{1/2\alpha}$ (min) | $t_{1/2\beta}$ (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| J124 | 0.0455 | 0.0086 | 0.0099 | $7.28 \times 10^{-5}$ | 0.0155 | 0.091 | 0.079 | 4.2 | 12.4 | 85.9 |
| J128 | 0.0272 | 0.0067 | 0.0143 | $1.25 \times 10^{-4}$ | 0.0079 | 0.150 | 0.070 | 4.1 | 18.3 | 67.5 |
| L962 | 0.0341 | 0.0094 | 0.0116 | $6.39 \times 10^{-5}$ | 0.0116 | 0.096 | 0.078 | 3.3 | 14.9 | 81.6 |
| L976 | 0.0516 | 0.0145 | 0.0216 | NA | NA | 0.055 | 0.037 | 2.8 | 9.6 | 45.0 |
| Mean ± SD | 0.0396 ± 0.0095 | 0.0098 ± 0.0029 | 0.0144 ± 0.0045 | $8.72 \times 10^{-4} \pm 2.69 \times 10^{-5}$ | 0.0117 ± 0.0031 | 0.098 ± 0.034 | 0.066 ± 0.017 | 3.6 ± 0.6 | 13.8 ± 3.2 | 70.0 ± 16.0 |

A three-compartment model (central, peripheral, and CSF) is assumed

by dividing the AUMC by the AUC. The penetration of drug into the CSF was calculated by the ratio of the AUC of the CSF to the AUC of the plasma.

Model dependent data analysis was done using ADAPT II [13]. An overall three-compartment model consisting of two compartments for the plasma (one peripheral and one central compartment) and one for the CSF was used to simultaneously fit the plasma and CSF concentration-time data (Fig. 2). Akaike's information criterion was used to select the best model. In the final model the CSF volume of distribution was fixed at 10 ml, which is the approximate volume of CSF in the rhesus monkey. The other volumes of distribution and the model rate constants were directly estimated from ADAPT II for each animal. The distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were calculated from the model rate constants.

### Evaluation for toxicity

Clinical laboratory studies including complete blood counts, electrolytes, liver function tests, and renal function tests were obtained on a weekly basis for a minimum of 3 weeks after each pemetrexed infusion. Animals were also observed on a daily basis for a minimum of 3 weeks after each infusion for any evidence of clinical toxicity.

## Results

Results of the pharmacokinetic data analysis from the individual experiments are given in Tables 1, 2 and 3. Figure 3 shows the plasma and CSF concentration-time curves as logarithmic means of all the experiments.

The model independent plasma pharmacokinetic results are reported in Table 1. Model independent results include $C_{max}$, AUC, $Cl_{TB}$, MRT, $Vd_{ss}$. As the table illustrates, the variation between subjects of the model independent parameters is relatively small. As the table also shows at a dose of 20 mg/kg a mean $C_{max}$ of 247 ± 540 μM was achieved with a mean AUC of 141 ± 17 μM h. The MRT was 60 ± 7.5 min.

Akaike's information criterion was used to show that the overall three-compartment model (two plasma compartments consisting of a one central and one peripheral compartment plus a CSF compartment) was superior to alternative models. The plasma pharmacokinetic parameters derived with the final model are given in Table 2. The disappearance of the drug from the plasma was described by a mean distribution half-life ($t_{1/2\alpha}$) of 13.8 ± 3.2 min and an elimination half-life

**Table 3** CSF pharmacokinetic parameters (model independent and model dependent) of pemetrexed after a single intravenous infusion to non-human primates

| Animal | $C_{max}$ (μM) | AUC (μM h)$_{0 \rightarrow \infty}$ | MRT (min) | $AUC_{csf}/AUC_{plasma}$ (%) |
|---|---|---|---|---|
| J124 | 0.17 | 0.42 | 116 | 0.36 |
| J128 | 0.47 | 2.08 | 199 | 1.58 |
| L962 | 0.15 | 0.52 | 163 | 0.33 |
| L976 | na | na | na | na |
| Mean ± SD | 0.26 ± 0.15 | 1.01 ± 0.76 | 159 ± 34 | 0.76 ± 0.59 |

($t_{1/2\beta}$) of 70.0 ± 16.0 min. The volume of distribution of the central plasma compartment ($V_1$) was 0.098 ± 0.034 l/kg and the volume of distribution of the peripheral plasma compartment ($V_2$) was 0.066 ± 0.017 l/kg. The clearance from the central compartment ($Cl_{central}$) was 3.6 ± 0.6 ml/min/kg.

The CSF pharmacokinetic results are in Table 3. As the table shows, the mean CSF maximum concentration was 0.26 ± 0.15 μM, which was less than 1% of the peak plasma concentration. Peak CSF concentrations were achieved 40–71 min after the start of the infusion. The ratio of CSF AUC to plasma AUC was less than 2% in all three monkeys from which CSF samples were available (range 0.33–1.58%). The MRT for the CSF was 159 ± 34 min. Figure 3 shows plasma and CSF concentrations of pemetrexed as logarithmic means for all animal experiments.

The protein binding of pemetrexed at a concentration of 1,600 ng/ml was 86% in non-human primate plasma and 81% in human plasma. The protein binding of pemetrexed at a concentration of 30 ng/ml was 100% in non-human primate plasma versus and 94% in human plasma.

Pemetrexed was well tolerated in all four animal subjects. There were no significant acute or chronic hematologic or other organ toxicities after a single intravenous dose of 20 mg/kg.

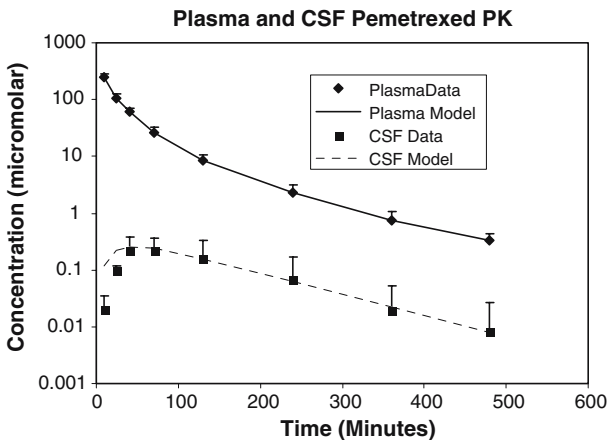


**Fig. 3** Plasma and CSF concentration–time curves of pemetrexed following an i.v. dose of 20 mg/kg

## Discussion

Plasma and CSF pharmacokinetics of pemetrexed were studied in a non-human primate model that has previously been shown to be predictive of CSF drug penetration in humans [7]. The simultaneous plasma and CSF pharmacokinetics were best described by a three-compartment model consisting of two plasma compartments (one peripheral and one central) and one CSF compartment (Fig. 2). The disappearance of pemetrexed from the plasma was best described by a two plasma compartment open model with a rapid distribution half-life of 13.8 ± 3.2 min and a terminal half-life of 70.0 ± 16.0 min.

Pharmacokinetic studies in humans have determined the terminal half-life of pemetrexed in plasma to be 1.4–3.9 h [4, 6, 8, 9]. The non-human primate model demonstrated a minimally shorter half-life (~ 1 h), which is consistent with the slightly faster clearance of pemetrexed in non-human primates (3.6 ± 0.6 ml/min/kg) versus humans (1.2–2.6 ml/min/kg, assuming a 1.7 $m^2$ adult) [4, 6, 8, 9].

The $IC_{50}$ for pemetrexed following a 72 h exposure was 0.016 μM (1.15 μM h) in the CCRF-CEM human leukemia cell line [14] and ranged from 0.017 to > 50 μM (1.22 to > 3,600 μM h) in various human gastric cancer cell lines [5]. The pemetrexed exposure in the non-human primate CSF was 1.01 ± 0.76 μM h following a dose of 400 mg/$m^2$. Since the standard dose of pemetrexed in adults is 500 mg/$m^2$ and the maximum tolerated dose in pediatrics is 1,910 mg/$m^2$ [11], it is quite likely that the exposure required for cytotoxicity is achievable in some malignancies. Furthermore, based on the results of our protein binding studies, it is apparent that the fraction of free drug is humans is slightly higher than non-human primates. This suggests that the CSF penetration in humans may be slightly higher than in non-human primates.

The CSF penetration of pemetrexed was less than 2% (range 0.33–1.58%). The MRT for drug in the CSF was slightly longer than the plasma but within the same order of magnitude and likely not clinically significant given the drug's poor penetration. The CSF penetration of pemetrexed is similar to the CSF penetration of standard dose methotrexate in non-

human primates and humans [2], and similar to the novel folate analogue raltitrexed [15]. From a clinical perspective, the low CSF penetration of pemetrexed suggests that systemic administration of pemetrexed alone may have some activity against leptomeningeal disease. However, similar to anti-metabolites such as methotrexate, systemic administration of pemetrexed as a single agent is unlikely to cure leptomeningeal metastases. However, because pemetrexed has preclinical activity against leukemia and a wide variety of solid tumors, regional administration may be an attractive alternative to overcoming the limited CSF penetration. Finally, the limited penetration of drugs such as pemetrexed across an intact blood brain barrier does not preclude their potential activity in CNS tumors or metastases with disrupted blood brain barriers.

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