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# A slow-release methotrexate formulation for intrathecal chemotherapy

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**Abstract.** Optimal anticancer treatment with cell cycle phase-specific antimetabolites requires a sustained maintenance of cytotoxic drug levels. However, drugs that can be administered intrathecally have short half-lives in cerebrospinal fluid (CSF) and require repeated administrations by lumbar punctures, which are painful and inconvenient. Implantable pumps are expensive and require surgery. In a rat model, a lipid-based formulation of methotrexate (Depo/methotrexate) was tested for extended maintenance of therapeutic CSF concentration after a single injection. The half-life of methotrexate in CSF after an intracisternal injection of Depo/methotrexate was 5.4 days compared to 0.30 days for unencapsulated methotrexate. This 18-fold increase in methotrexate half-life may make Depo/methotrexate useful for intrathecal chemotherapy of neoplastic meningitis.

## Introduction

Methotrexate is a cell cycle phase-specific drug that kills cancer cells only when they are dividing [6]. Since cancer cells grow more slowly in cerebrospinal fluid (CSF) [10], maintenance of a cytotoxic level of drug in the environment of cancer cells for an extended period of time is essential for optimal therapy. However, methotrexate is cleared rapidly from the CSF [12]. At present, sustained cytotoxic drug concentrations can only be achieved by repeated intrathecal or intraventricular injections [3]. These repeated administrations are uncomfortable and risky, and the implantable pumps are expensive and require surgery. Therefore, a slow-release depot preparation of methotrexate that will maintain therapeutic drug concentrations in the CSF for an extended period is needed for intracavitary chemotherapy of neoplastic meningitis.

We have used a lipid-based drug delivery system, DepoFoam [7], as a slow-releasing depot for methotrexate. Depo/methotrexate is composed of microscopic particles that enclose multiple nonconcentric aqueous chambers in which methotrexate has been encapsulated. The lipid particles themselves are made from nontoxic lipids identical to those found in cell membranes. We report here a pharmacokinetic study of Depo/methotrexate given by intracisternal injection in a rat model.

## Material and methods

*Animals.* Male Sprague-Dawley rats, 50–90 days of age, weighing 350–410 g, were obtained from Simonsen Laboratories (Gilroy, Calif.). Animals were housed in individual cages, given free access to food and water, and maintained in accordance to the regulations of the UCSD Animal Research Committee and those of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

*Surgical instruments and equipment.* All surgical instruments were purchased from Roboz. Stereotaxic apparatus was obtained from David Kopf Instruments, Tujunga, Calif.

*Material used in preparation of Depo/methotrexate.* Sodium salt of methotrexate for injection was obtained from the National Cancer Institute; 2-hydroxypropyl-β-cyclodextrin was purchased from Pharmatec, Inc. (Alachua, Fla.); dioleoyl lecithin, dipalmitoyl phosphatidylglycerol, and cholesterol were acquired from Avanti Polar Lipids, Inc. (Birmingham, Ala.); triolein and free-base L-lysine were procured from Sigma (St. Louis, Mo.); and nanograde chloroform was obtained from Mallinckrodt (Paris, Ky.). All reagents were used without further purification.

*Synthesis of Depo/methotrexate.* Depo/methotrexate was prepared using a previously published method for encapsulating cytarabine [7] with some modifications. For each batch of Depo/methotrexate, the discontinuous aqueous phase consisted of 2-hydroxypropyl-β-cyclodextrin solution (100 mg/ml), HCl (0.1 N), and methotrexate (10 mg/ml). One

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milliliter of the discontinuous aqueous phase was added into a 1-dram vial containing 13.9 μmol dioleoyl lecithin, 3.15 μmol dipalmitoyl phosphatidylglycerol, 22.5 μmol cholesterol, 2.7 μmol triolein, and 1 ml chloroform. The vial was attached horizontally to the head of a vortex mixer and shaken at maximum speed for 6 min. Half of the resulting "water-in-oil" emulsion was expelled rapidly through a narrow-tip Pasteur pipette into each of two 1-vials, each containing 2.5 ml water, glucose (32 mg/ml) and free-base lysine (40 mM). Each vial was then shaken on the vortex mixer for 5 s at maximum speed to form chloroform spherules. The chloroform spherule suspensions in the two vials were transferred into a 250-ml Erlenmeyer flask containing 5 ml water, glucose (32 mg/ml), and free-base lysine (40 mM). A stream of nitrogen gas at 7 l/min was used to evaporate the chloroform over a 10–15 min period at 37° C. The DepoFoam particles were then isolated by centrifugation at 600 *g* for 5 min and washed three times with 0.9% NaCl solution.

*Intrathecal pharmacokinetic studies.* Rats were anesthetized with ketamine HCl (90 mg/kg) and acetopromazine maleate (2.2 mg/kg), injected intramuscularly, and mounted in a conventional stereotaxic frame. Using a no. 15 blade, a midline cutaneous incision approximately 1 cm in length was made from the occipital crest to just behind the ears. The muscle ligament along the occipital crest at the skull was detached with a scalpel for 4 mm on either side of the midline. Using both the sharp and blunt ends of a periosteal elevator, the muscle from the occipital bone was freed down to the atlanto-occipital membrane. A retractor was placed in the incision to draw the muscle aside and obtain a clear view of the atlanto-occipital membrane. Either 20 μl unencapsulated methotrexate or 20 μl Depo/methotrexate in 0.9% NaCl, both containing 100 μg (0.22 μmol) methotrexate, was then injected over 20 s via a 30-gauge needle through the membrane. The needle was withdrawn, the skin was sutured with 3–0 silk, and the animal was given 10 ml lactated Ringer's solution subcutaneously for hydration.

At appropriate time points after injection, the atlanto-occipital membrane was again exposed under anesthesia and a sample of CSF ranging from 30 to 60 μl was obtained through a 19-gauge needle. CSF samples were obtained from three rats at each time: at 1 min and at 4, 24, and 48 h after injection in the unencapsulated methotrexate group and at 1 min and at 1, 3, 7, 14, and 21 days after injection in the Depo/methotrexate group. The CSF samples from the Depo/methotrexate group were diluted with 70 μl 0.9% NaCl solution and then immediately centrifuged in an Eppendorf Microfuge for 1 min to separate a supernatant containing released free methotrexate from a pellet containing encapsulated methotrexate. Next, 50 μl of methanol and 50 μl of sterile water were sequentially added to the pellet and vortexed to break the Depo/methotrexate particles. The CSF samples were then kept frozen at –20° C until analyzed using a high-performance liquid chromatography (HPLC) system as described below.

After CSF sampling, the animals were sacrificed with an overdose of ketamine (90 mg/kg) and acetopromazine (20 mg/kg) injected intraperitoneally. Blood samples were obtained via cardiac puncture and thorough exsanguination was performed. The plasma was separated and kept frozen at –20° C until analyzed by Emit methotrexate assay (Syva Company, Palo Alto, Calif.) on COBAS Fara instrument (Roche Diagnostic Systems, Montclair, N.J.). The Emit assay was a homogeneous enzyme immunoassay technique with a limit of sensitivity of 0.02 μM. The calvarium was then exposed and carefully removed with a bone rongeur. The entire content of the cranial compartment was collected by scooping out the exposed brain with a spatula and washing the cranial vault thoroughly with distilled water. The spinal compartment content was then collected separately: the spinal cord was extruded forward into the cranial vault by pushing distilled water rapidly through a 19-gauge needle inserted into the lower lumbar spinal canal at a point 2.5 cm rostrad to the origin of the tail. The empty spinal canal was washed out thoroughly with distilled water to complete collection of methotrexate in the spinal canal. The cranial compartment samples were analyzed separately from the spinal canal samples. Both tissue samples were homogenized with water using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The amount of methotrexate in the spinal compartment was calculated by adding in the amount from the cisternal CSF sample. The homogenized samples of brain or spinal cord were analyzed with HPLC after extraction according to a previously described method [1]. Briefly, in a glass centrifuge tube, a 500 μl aliquot of homogenate, 100 μl theophylline aqueous solution (internal standard, 2.0 mg/ml), 250 μl trichloroacetic acid solution (10% in water), and 250 μl glacial acetic acid were placed and mixed. Then, methotrexate free acid was extracted with 5 ml ethyl acetate. Ethyl acetate organic phase was decanted and evaporated under nitrogen at 60° C. The extracted residue was dissolved in 200 μl of mobile phase and 100 μl of the resulting solution was injected into HPLC. Mobile phase consisting of $H_3PO_4$ (10 mM): $KH_2PO_4$ (10 mM): methanol in 180:540:280 ratio (final pH = 3) was pumped at a flow rate of 1 ml/min with a Waters model 510 pump through a Beckman ultrasphere ODS 5 μm × 4.6 mm × 25 cm column. Methotrexate was detected at 303 nm with a Waters Model 490 programmable multiwavelength detector. The retention times of theophylline and methotrexate were 5.2 and 7.2 min, respectively. The limit of detection was 5 pmol of methotrexate injected.

*Pharmacokinetic analysis.* The RSTRIP computer program (MicroMath Scientific Software, Salt Lake City, Utah) was used to perform the pharmacokinetics analysis. The area under the curve (AUC) was determined by linear trapezoidal rule up to the last measured concentration and extrapolated to infinity.

## Results

### *Depo/methotrexate characterization*

The average volume-weighted diameter of Depo/methotrexate was found to be 14.1 ± 3.4 (± SD) μm. Encapsulation efficiency was 64.5 ± 6% ($n = 6$) and captured volume was 12.9 ± 1.0 μl/μmol of lipids. Storage of Depo/methotrexate at 4° C in 0.9% NaCl solution resulted in less than 5% release of methotrexate after 4 months.

### *CNS Pharmacokinetics*

Figures 1 and 2 depict the central nervous system (CNS) pharmacokinetics (in terms of CSF concentration and CNS amount) of Depo/methotrexate and unencapsulated methotrexate. After intracisternal injection of Depo/methotrexate, the CSF concentration of free methotrexate reached a maximum on day 1 and then decreased in a biexponential fashion with initial and terminal half-lives of 0.41 and 5.4 days, respectively. The terminal half-life was 18 times longer than that for unencapsulated methotrexate.

Following injection of Depo/methotrexate, the total amounts of drug within CNS decreased with a half-life of 9 days compared to 0.03 days for unencapsulated methotrexate. At the end of the 21-day period, 18% of the methotrexate remained within the CNS after Depo/methotrexate injection.

Pharmacokinetics parameters for methotrexate and Depo/methotrexate within the CNS are summarized in Table 1. Maximum concentration of free methotrexate after Depo/methotrexate administration was about 70 times lower than that after administration of unencapsulated methotrexate.

The proportions of the total amount of methotrexate within the cranial compartment were 12 ± 8%, 65 ± 11%, 51 ± 40%, and 65 ± 36%, respectively, at 1 min and 7, 14, and 21 days after injection of Depo/methotrexate and

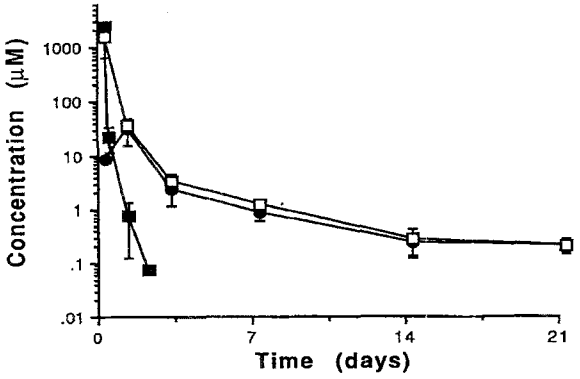


**Fig. 1.** Concentrations of methotrexate in CSF after intracisternal injection of 100 μg (0.22 μmol) methotrexate as Depo/methotrexate (●, free; □, total) or unencapsulated methotrexate (■). Each data point represents mean and standard deviation from three rats.

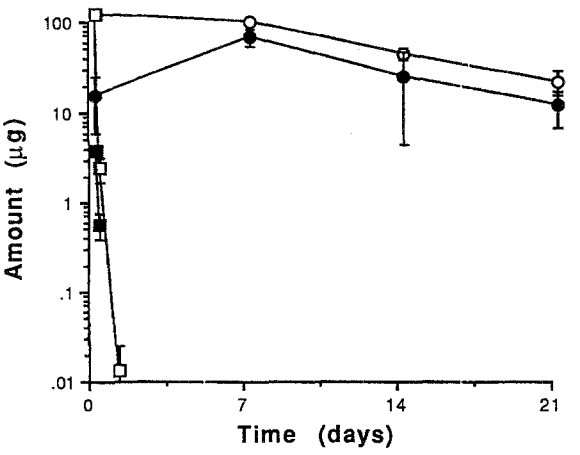


**Fig. 2.** Amount of methotrexate remaining within the CNS after intracisternal injection of 100 μg methotrexate as Depo/methotrexate (○, total within CNS; ●, within cranial compartment) or unencapsulated methotrexate (□, total; ■, cranial). Each data point represents mean and standard deviation from three rats

4±1% and 23±1%, respectively, at 1 min and 4 h after injection of unencapsulated methotrexate.

Analysis of plasma concentrations showed undetectable levels of methotrexate (limits of detection being 0.02 μM) except at one time point after unencapsulated drug (4 h after intracisternal injection: 0.11±0.02 μM).

*Toxicities*

No abnormalities were observed in the behavior of rats given injections of Depo/methotrexate over the period of the study. Three rats injected with Depo/methotrexate gained weight from 343±5 to 383±19 g over the 3 weeks, while control rats without any injections or surgical interventions grew from 340±1 to 400±12 g.

## Discussion

The encapsulation of methotrexate in DepoFoam resulted in a 18-fold increase in the terminal half-life of free methotrexate from the CSF. The free methotrexate concentrations stayed above an arbitrary 0.5 μM (the minimal cytotoxic concentration estimated from studies in vitro [5]) for 7–14 days after a single injection of Depo/methotrexate. In contrast, the duration was about 1 day for the unencapsulated drug.

The AUC of free concentrations for the Depo/methotrexate group was one third of that for the unencapsulated group. This may be attributable to saturation of the methotrexate CSF clearance mechanism [4, 11] when high free methotrexate concentrations occur in the unencapsulated group. A second possibility is that a higher fraction of the free methotrexate penetrates into the brain and spinal cord parenchyma by extended exposure and thus a smaller fraction remains in the CSF. Yet another possibility is that the AUC for the Depo/methotrexate was understimated due to the sampling schedule.

The comparison of the total amount of methotrexate and the amount within the cranial compartment (Fig. 2) showed good distribution of Depo/methotrexate into both spinal and cranial compartments after intracisternal injection. For example, at day 21 the amount of methotrexate within the cranial compartment was 65±36% of the total (cranial plus spinal) amount. However, a large fraction of methotrexate in the cisternal sample was in the form of free drug after the first day of injection with Depo/methotrexate. We speculate that a high density of Depo/methotrexate particles relative to the CSF results in settling of Depo/methotrexate particles by gravity away from the cisternal CSF, whereas the released free methotrexate is free to diffuse.

Other investigators have previously reported encapsulation of methotrexate into a variety of drug delivery systems [9, 13, 14]. However, the release rates of methotrexate were found to be rather rapid and the previous encapsulations did not result in any major changes in pharmacokinetics. Kimelberg et al. [9] reported the half-life of the liposomal methotrexate preparation in the CSF to be extremely short (less than 1 h), not significantly different from the unencapsulated drug. The extended release of methotrexate from Depo/methotrexate, both in vitro and in vivo, indicates that DepoFoam may be useful as a drug depot for methotrexate.

**Table 1.** Pharmacokinetic parameters of methotrexate in the CNS after a 100-μg injection

| | Methotrexate | Depo/methotrexate | |
|---|---|---|---|
| | | Free | Total |
| $C_{max}$ (μM) | 1751±302 | 23.7±11.7 | 1133±631 |
| Conc. $t_{1/2}$ α (days) | 0.024 | 0.41 | 0.18 |
| Conc. $t_{1/2}$ β (days) | 0.30 | 5.4 | 4.0 |
| AUC (μM × days) | 154.3 | 50.5 | 624.2 |
| Amount $T_{1/2}$ (days) | 0.03 | NA | 9.0 |

$C_{max}$, maximum CSF concentration; $t_{1/2}$, half-life; AUC, area under the curve; NA, not applicable

There is concern regarding possible increased neurotoxicity with slow-release formulations [2]. However, it is not clear which is more neurotoxic: high concentration or extended low concentration. This was tested in a small clinical trial in humans [3] comparing a bolus dose and "C × T" dosing. It appears that neurotoxicity could be avoided by reducing the peak free methotrexate concentration while maintaining efficacy [3]. With Depo/methotrexate, it is possible that neurotoxicity can be reduced by keeping most of the initial bolus of methotrexate within DepoFoam particles, and yet tumor kill enhanced by maintaining the free methotrexate to just above the minimum cytotoxic concentration for an extended period. The results of this pharmacokinetics study offer the possibility of less frequent intra-CSF administration for the prophylaxis and treatment of leptomeningeal leukemia or carcinomatosis in humans. With a similar slow-release formulation of cytarabine (Depo/ara-C) [8], we have recently completed a pharmacokinetics and dose-ranging clinical trial in humans at UCSD Cancer Center. Initial clinical testing of Depo/methotrexate in humans is planned.

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