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An extended-release formulation of methotrexate for subcutaneous administration

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Abstract. Methotrexate is a cell-cycle phase-specific antimetabolite that requires prolonged exposure for optimal efficacy. However, the in vivo half-life is short, and repeated administrations are required for optimal efficacy. Methotrexate was encapsulated into a lipid-based drug-delivery system to create an extended-release formulation (Depo/methotrexate) for subcutaneous administration. Pharmacokinetics and efficacy studies were done in BDF1 mice. Depo/methotrexate increased the methotrexate plasma half-life by a factor of 190, from 0.53 to 100 h. Plasma peak levels of the encapsulated drug were 120-fold lower than those of unencapsulated methotrexate, whereas the AUCs (areas under the concentration-time curve) were similar. As a consequence of the extended drug release, the single-dose potency of methotrexate against the L1210 leukemia model was increased by a factor of about 130 without producing significant changes in the therapeutic index. In conclusion, Depo/methotrexate appears to have potential usefulness as an extended-release formulation of methotrexate.

Introduction

Methotrexate is an important cytotoxic agent with a broad spectrum of activity against a variety of malignancies such as acute lymphocytic leukemia, osteosarcoma, non-Hodgkin's lymphoma, choriocarcinoma, head and neck cancer, and breast cancer as well as against nonneoplastic conditions such as severe psoriasis and rheumatologic diseases [5]. For this drug, as is the case for other cell-cycle phase-specific agents, a prolonged duration of drug exposure is necessary for optimal efficacy because the drug kills only those cells that are actively dividing [15]. As methotrexate has a short half-life in vivo, an extended-release formulation is needed.

We have explored a lipid-based drug-delivery system to synthesize an extended-release depot for methotrexate, Depo/methotrexate [3]. Depo/methotrexate consists of microscopic particles of lipid bilayer membrane that enclose multiple nonconcentric aqueous chambers, into which methotrexate is encapsulated [9]. The lipid particles themselves are made from nontoxic lipids identical to those found in cell membranes. In this article, we report the results of pharmacokinetics and efficacy studies of Depo/methotrexate delivered subcutaneously in an animal model.

Materials and methods

Materials. Methotrexate was obtained from the National Cancer Institute (USA); 2-hydroxypropyl-β-cyclodextrin was purchased from Pharmatec (Alachua, Fla.); dioleoyl lecithin, dipalmitoyl phosphatidylglycerol, and cholesterol were acquired from Avanti Polar Lipids (Birmingham, Ala.); triolein and free-base L-lysine were procured from Sigma (St. Louis, Mo.); and nanograde chloroform was obtained from Malinckrodt (Paris, Ky.). All reagents were used without further purification. The vortex mixer was obtained from American Scientific Products (catalogue number S8223-1, McGaw Park, Ill.). BDF1 and DBA/2J mice were supplied by Simonsen Laboratories (Gilroy, Calif.). The L1210 leukemia was maintained by serial intraperitoneal passage in female DBA/2J mice.

Synthesis of Depo/methotrexate. Depo/methotrexate was prepared with modifications of the previously published method for encapsulating arabinofuranosylcytosine (ara-C) [8]. For each batch of Depo/methotrexate, the discontinuous aqueous phase consisted of 2-hydroxypropyl-β-cyclodextrin (100 mg/ml), HCl (0.1 *N*), and methotrexate (10 mg/ml). In all, 1 ml of the discontinuous aqueous phase was added into a 1-dram vial

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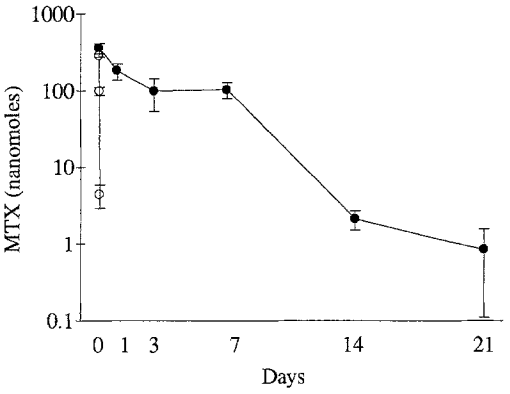

Fig. 1. Amount of Std/methotrexate (*open circles*) and Depo/methotrexate (*filled circles*) recovered from the s.c. tissues. Note the semilog scale

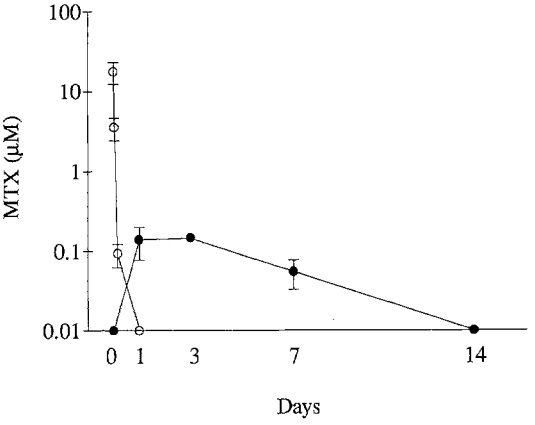

Fig. 2. Plasma concentrations of methotrexate following s.c. injection of Std/methotrexate (*open circles*) and Depo/methotrexate (*filled circles*) Note the semilog scale

Table 1. Pharmacokinetic parameters

	Std/methotrexate	Depo/methotrexate
Injection site:		
Amount $t_{1/2}$ (*h*)	0.16 ± 0.01	49.7 ± 3.5
Plasma:		
C_{max} ± SD (μ*M*)	17 ± 5	0.14 ± 0.06
Conc. $t_{1/2}$ (h)	0.53 ± 0.01	100 ± 5
AUC (μ*M* h)	18 ± 4	28 ± 9

$t_{1/2}$, Half-life; C_{max}, peak concentration; AUC, area under the concentration-time curve

containing 13.9 μmol of dioleoyl lecithin, 3.15 μmol of dipalmitoyl phosphatidylglycerol, 22.5 μmol of cholesterol, 2.7 μmol of triolein, and 1 ml of chloroform. The vial was attached horizontally to the head of the vortex mixer and shaken at maximal 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 vials (1-dram) containing 2.5 ml of water, glucose (32 mg/ml), and free-base lysine (40 m*M*). Each vial was then shaken on the vortex mixer for 5 s at maximal speed to form chloroform spherules. The chloroform-spherule suspensions in the two vials were transferred into a 250-ml Erlenmeyer flask containing 5 ml of water, glucose (32 mg/ml), and free-base lysine (40 m*M*). A stream of nitrogen gas flowing at 7 l/min was used to evaporate the chloroform over a period of 10–15 min at 37° C. The Depo/methotrexate particles were then isolated by centrifugation at 600× *g* for 5 min and washed thrice with 0.9% NaCl solution.

Pharmacokinetics studies. Subcutaneous (s.c.) pharmacokinetics studies were done using male BDF1 mice weighing 20–25 g. Mice were injected s.c. into the center of the abdominal skin with 10 mg/kg (22 μmol/kg) of unencapsulated standard methotrexate (Std/methotrexate) or Depo/methotrexate in 200 μl of 0.9% NaCl solution using a 30-gauge hypodermic needle. Blood samples were obtained from the jugular vein of animals under anesthesia at time points 0, 0.25, 1, and 4 h for the Std/methotrexate group and at time points 0, 1, 3, 7, 14, and 21 days for the Depo/methotrexate group. At each time point, three animals were killed. The plasma was separated and kept frozen at –20° C until analyzed by the Emit methotrexate assay (Syva Company, Palo Alto, Calif.) on a COBAS Fara instrument (Roche Diagnostic Systems, Montclair, N.J.). The Emit assay is a homogeneous enzyme-immunoassay technique with a limit of sensitivity of 0.02 μ*M*.

A full thickness of the abdominal wall tissue, including the entire skin and the underlying peritoneal membrane, was then excised from the costal margin to the inguinal area from one flank to another. The entire tissue specimen was homogenized after the addition of at least 20 ml of distilled water in a Polytron homogenizer (Brinkmann Instruments, Westbury, N.J.). The homogenate was sonicated for 60 s with a probe sonicator (maximal setting, Biosonic IV; VWR Scientific, San Francisco, Calif.) and filtered through a YMT ultrafiltration membrane (Amicon Corp., product number 4104). All the samples were kept at –20° C until assayed by high-performance liquid chromatography (HPLC). The RSTRIP program (MicroMath Scientific Software, Salt Lake City, Utah) was used to perform the curve fitting. A single-compartment model was used. The area under the concentration-time curve (AUC) was determined by linear trapezoidal rule up to the last measured concentration and then extrapolated to infinity.

HPLC assay. A mobile phase consisting of H_3PO_4(10 m*M*):KH_2PO_4(10 m*M*):methanol at a 162:488:350 ratio (pH 3) was pumped at a flow rate of 1 ml/min with a Waters Model 510 pump through a Beckman ultrasphere ODS 5-μ 4.6-mm × 25-cm column. Methotrexate was detected at 303 nm by a UV Waters 490 programmable Multiwave-length Detector. The retention time of methotrexate was 5 min and the detection limit was 5 pmol (injected quantity).

Toxicity and efficacy studies. BDF1 mice were injected with 10^6 L1210 cells into the peritoneal cavity on day 0 and were treated s.c. with a single dose of Std/methotrexate or Depo/methotrexate suspended in 0.9% NaCl on day 1. Five animals were in each group, except for the control (given 0.9% NaCl alone), where ten animals were used. Each animal was observed for survival. The median duration of survival was used to calculate the increase in life span (ILS) according to the formula:

$$ILS = (T - C)/C \times 100\%,$$

where *T* is the median duration of survival for the treated groups and *C* is that for the control groups.

Results

Characteristics of Depo/methotrexate

The average volume-weighted diameter of Depo/methotrexate was 14.1 ± 3.4 (± SD) μm. The efficiency of encapsulation was 64.5% ± 6% (n = 6) and the capture volume was 12.9 ± 1.0 μl/μmol of lipid used. The percentage of drug release in 0.9% NaCl at 4 months was <5% at 4° C. In human plasma at 37° C, the half-life of drug release was 40 days.

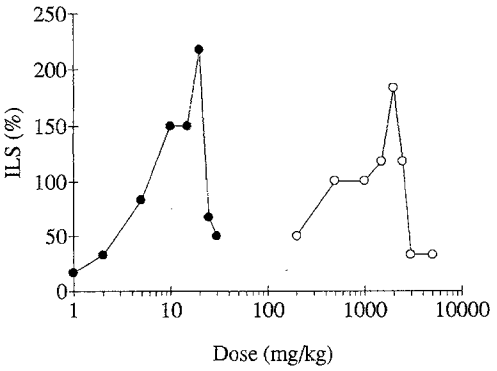

Fig. 3. Percentage of increase in life span (ILS) as a function of the log of the delivered dose for Std/methotrexate (*open circles*) and Depo/methotrexate (*filled circles*)

Subcutaneous pharmacokinetics

The pharmacokinetic parameters are summarized in Table 1. After an s.c. injection, the total amount of methotrexate in the skin decreased exponentially with a half-life of 0.16 h for Std/methotrexate and 49.7 h for Depo/methotrexate (Fig. 1). In plasma, the half-lives were 0.53 h for Std/methotrexate and 100 h for Depo/methotrexate. The peak plasma levels were $17 \pm 5 \mu M$ (SD) at 15 min for Std/methotrexate and 0.14 ± 0.06 μM (SD) at day 3 for Depo/methotrexate (Fig. 2).

Efficacy studies

Figure 3 shows the ILS curves in a murine L1210 model. The maximal efficacy (ILS_{max}) was 183% for Std/methotrexate and 217% for Depo/methotrexate ($P = 0.5$, Mann-Whitney U-test). The relative potency of single-dose Depo/methotrexate versus Std/methotrexate was 130 (determined by the PHARM/PCS program, MicroComputer Specialists, Philadelphia, Pa.). The dose lethal to 50% of the mice (LD_{50}) was calculated after probit transformation. The LD_{50} for a single dose of Std/methotrexate was 2650 mg/kg and that for Depo/methotrexate was 24 mg/kg, resulting in a ratio of 110.

Discussion

Methotrexate has previously been encapsulated into other drug-delivery systems [11, 16, 17]. However, the drug-release rates were rather rapid and resulted in short half-lives in vivo without any major changes in pharmacokinetics and pharmacodynamics.

Depo/methotrexate may be useful in extended-release drug delivery of methotrexate for several reasons. Depo/methotrexate particles can be synthesized in large average sizes, which can decrease their uptake into lymphatics and the systemic circulation after injection into body cavities or into tissue spaces such as the s.c. space [8 – 10]. Their large size may also inhibit uptake into macrophages. Furthermore, their sponge-like internal structure [7] results in efficient encapsulation into aqueous internal chambers, stability in storage, and extended release in vivo. Clinical trials in progress at UCSD Cancer Center with another antimetabolite, cytarabine, encapsulated into a similar lipid-based drug-delivery system have demonstrated extended release in humans [10].

In this study we used Depo/methotrexate to increase the half-life of a cell-cycle phase-specific drug in an animal model. The results showed that the half-life in plasma was 190-fold longer and the peak plasma concentration was 120-fold lower as compared with those of Std/methotrexate, whereas the AUC was essentially unchanged. As a consequence of the significant modifications of the pharmacokinetics, the drug's potency was increased by a factor of 130 and the LD_{50} was decreased by a factor of 110. These changes in potency and LD_{50} indicate a lack of significant change in the therapeutic index. The above findings confirm previous findings for cell-cycle phase-specific agents that the duration of tumor-cell exposure is a critical determinant of cytotoxicity and that longer drug exposures are much more cytotoxic [4, 13].

Although methotrexate can be given orally, parenteral administration has certain advantages. The absorption rate of methotrexate after oral administration is variable among patients and appears to be saturable [1, 6, 18]. In contrast, absorption of the drug after intramuscular (i.m.) or s.c. administration is much more predictable and complete, resulting in serum concentrations higher than those measured after an oral dose [2, 14, 18]. The variability of oral dosing can explain the higher relapse rate observed during the maintenance phase of acute lymphoblastic leukemia in children treated with oral methotrexate [12]. In conclusion, Depo/methotrexate appears to be useful as an extended-release vehicle for methotrexate delivery.

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