International Journal of Pharmaceutics, 79 (1992) 71–75

IJP 02623

Effects of leucovorin on the pharmacokinetics of methotrexate in rabbits

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(Received 19 July 1991)
(Accepted 23 August 1991)

Key words: Pharmacokinetics; Methotrexate; Leucovorin; Renal tubular secretion

Summary

The effects of leucovorin on the pharmacokinetics of methotrexate (MTX) were studied in rabbits. The renal tubular secretion of MTX appeared to be inhibited by administration of leucovorin, however, the efflux of MTX from the cell and/or re-entry of MTX into the cell did not seem to be affected by administration of leucovorin.

Methotrexate (MTX) is one of the most widely used antifolates in cancer chemotherapy for a variety of neoplasms. It exerts its cytotoxic effect by competitively inhibiting dihydrofolate reductase, the intracellular enzyme responsible for converting folic acid to reduced folate cofactors (Evans et al., 1986). Conventionally, MTX has been administered orally or parenterally in low doses (LDMTX therapy), i.e., 1–100 mg per m^2 of body surface area. The incidence of side effects after LDMTX therapy is less common and leucovorin is rarely administered in combination with MTX in LDMTX therapy. Recently, MTX has been administered in high doses (HDMTX therapy), i.e., 1–30 g per m^2 of body surface area, therefore, the administration of leucovorin is required to prevent severe toxicities, such as mucositis, pancytopenia, GI desquamation, and renal and hepatic dysfunctions.

In vitro kinetic studies on the uptake of MTX into the various cells have demonstrated that its transport might be mediated by two carrier systems, one with a low affinity and high capacity, and the other with a high affinity and low capacity (Shen and Azarnoff, 1978; Gewirtz et al., 1980; Schilsky et al., 1981). Although leucovorin has been widely used in HDMTX therapy, the effects of leucovorin on the pharmacokinetics of MTX appear not to have been studied thoroughly (Shen and Azarnoff, 1978; Nixon, 1979).

The purpose of this Note is to investigate the effects of leucovorin on the pharmacokinetics of MTX in rabbits.

15 healthy male New Zealand white rabbits (A–O, 1.75–2.85 kg) were anesthetized with 50–100 mg of intravenous (i.v.) ketamine (kindly supplied by Yu-Han Pharmaceutical Co., Seoul, Ko-

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rea). The carotid artery and jugular vein were catheterized with silastic tubing (Dow Corning, Midland, MI) for blood sampling and drug administration, respectively. The animals were allowed to recover from anesthetization for 4-5 h before the study. Urine samples were collected using a pediatric Foley catheter (Dover, Searl Medical Products, USA Inc., Dallas, TX) which was introduced into the urinary bladder.

MTX, 20 mg kg^{-1} (reconstituted with 0.9% NaCl injectable solution to make 50 mg per 3 ml, kindly supplied by Yu-Han Pharmaceutical Co.), was injected i.v. bolus in 10 s via the jugular vein of five rabbits (A–E, treatment I). Leucovorin, 20 mg kg^{-1} (3 mg per ml, kindly supplied by Lederle Laboratories, Pearl River, NY), was infused in 1 min before (rabbits, F–J, treatment II) and after (rabbits, K–O, treatment III) i.v. bolus administration of MTX (20 mg kg^{-1}). The time elapsed between MTX and leucovorin administration was 1 min. The midpoint of the injection of MTX was designated as zero time. Blood and urine collection methods were similar to those reported previously (Yoon et al., 1991).

MTX was quantitated by a slight modification (Lee et al., 1984) of an HPLC method developed earlier (Chen and Chiou, 1981). Sample preparation was simple: 2.5 vols of acetonitrile were added to the biological samples. After vortexing and centrifugation, 50 μl of the supernatant was injected directly onto the HPLC column.

Pharmacokinetic parameters, such as the area under the plasma concentration-time curve from time zero to time infinity (AUC), apparent volume of distribution at steady state (V_{ss}), time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, area under the first moment of the plasma concentration-time curve (AUMC), and mean residence time (MRT) were estimated according to standard methods (Riegelman and Collier, 1980; Yoon et al., 1991). The mean values of each clearance, $t_{1/2}$ and V_{ss} were calculated using the harmonic mean method (Chiou, 1979). The data were analyzed for statistical significance ($p < 0.05$) using analysis of variance.

The mean arterial plasma concentration-time curves of MTX from treatments I–III are shown

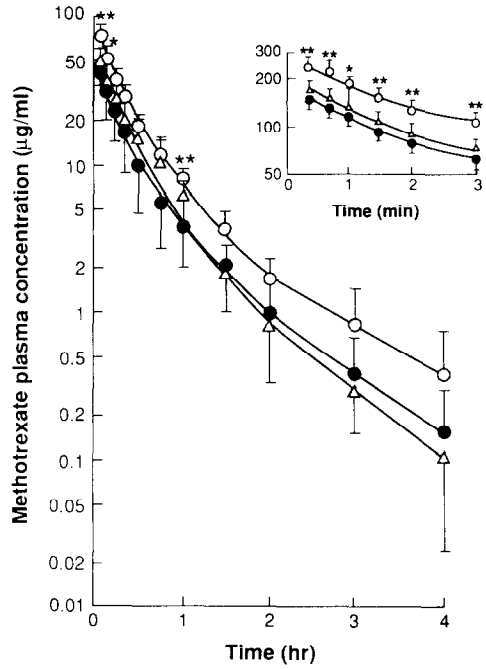

Fig. 1. Mean plasma concentration-time profiles of methotrexate from treatments I (●), II (○), and III (△). Inset shows the plasma concentration-time profiles of methotrexate during the first 3 min. Bars represent S.D. * $p < 0.05$, ** $p < 0.001$ when compared with the value from treatment I.

in Fig. 1, and the relevant pharmacokinetic parameters are listed in Table 1. After i.v. bolus dose, the plasma concentrations of MTX declined polyexponentially in all rabbits studied with mean terminal half-lives of 42.0, 47.9 and 26.4 min for treatments I–III, respectively. Similar mean terminal half-lives of 44.2, 48.9 and 38.8 min were also determined from the urinary excretion data (Fig. 2). The plasma concentrations of MTX from treatment II seemed to be higher than those from treatment I (Fig. 1) and resulted in a significantly lower value of CL (15.0 vs 8.80 ml min^{-1} kg^{-1}) than that from treatment I (Table 1). It should be noted that the mean plasma concentrations of MTX were determined for up to 4 h after the i.v. bolus dose (Fig. 1), and might be due to our assay sensitivity.

It is difficult to measure directly the tissue uptake of MTX in animals including humans. It appears that the most convenient pharmacokinetic parameter to express quantitatively the up-

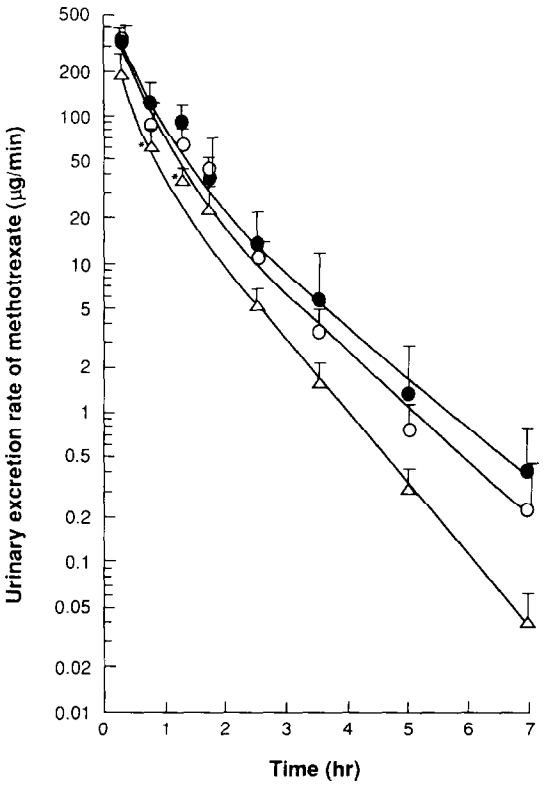

Fig. 2. Mean urinary excretion rate of methotrexate from treatments I (●), II (○) and III (△). Bars represent S.D. * $p < 0.05$ when compared with the value from treatment I.

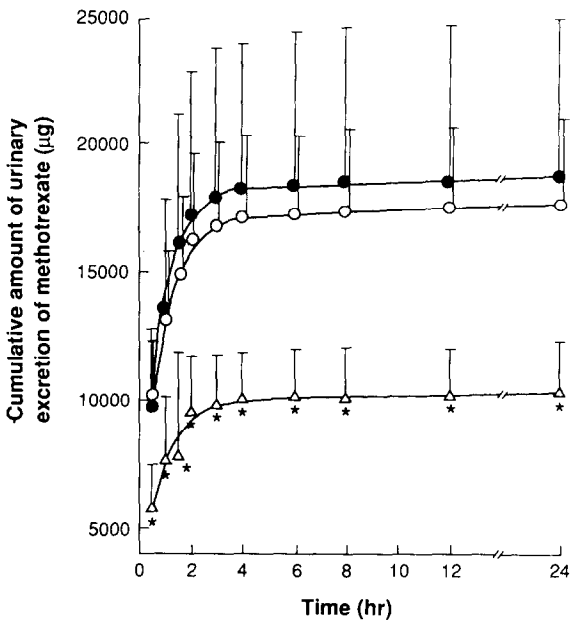

Fig. 3. Mean cumulative excretion of methotrexate from treatments I (●), II (○) and III (△). Bars represent S.D. * $p < 0.05$ when compared with the value from treatment I.

take of MTX into the cells is its apparent volume of distribution at steady state, V_{ss} (Lui et al., 1984). If the efflux of MTX from the cells and/or

TABLE 1

Mean (±S.D.) values of some pharmacokinetic parameters of methotrexate from treatments I–III (n = 5).

	Treatment I	Treatment II	Treatment III
AUC (μg min ml^{-1})	1340 ±447	2420 ±388 (0.0035)	1620 ±491 (0.36)
$t_{1/2}$ (min)			
Plasma data	42.0 ± 14.0	47.9 ± 24.4 (0.64)	26.4 ± 37.8 (0.42)
Urinary excretion data	44.2 ± 15.5	48.9 ± 10.1 (0.59)	38.8 ± 4.30 (0.47)
V_{ss} (ml kg^{-1})	411 ±287	264 ± 29.0 (0.098)	329 ± 83.4 (0.27)
CL (ml min^{-1} kg^{-1})	15.0 ± 5.93	8.08 ± 1.38 (0.014)	12.3 ± 3.18 (0.28)
CL_R (ml min^{-1} kg^{-1})	5.84 ± 3.63	2.64 ± 0.769 (0.025)	2.61 ± 1.32 (0.035)
CL_{NR} (ml min^{-1} kg^{-1})	8.10 ± 4.47	5.30 ± 0.932 (0.075)	9.53 ± 2.45 (0.77)
X_u (mg) [a]	18.5 ± 5.99	17.3 ± 3.38 (0.18)	10.1 ± 2.00 (0.00095)

Values in parentheses indicate p value when each value was compared with the value from treatment I.
[a] Amount of methotrexate excreted in 24-h urine.

re-entry of MTX into the cells is significantly affected by treatment with leucovorin in the present study, the values of V_{ss} from treatments II and III could be significantly different compared with that from treatment I. However, the V_{ss} values from treatments I–III were not significantly different although the mean values from treatments II and III appear to be decreased. It was found (Nahas et al., 1972) that the efflux of MTX from L1210 leukemia cells was increased by the addition of leucovorin to the media, and that MTX was a competitive inhibitor of leucovorin uptake into the cells. It was also suggested (Goldman, 1975) that 'quite-high' blood levels of leucovorin were required in order to accelerate the net efflux of MTX from the cell and to reduce the subsequent re-entry of MTX into the cell. Therefore, the above data suggested that the plasma concentrations of leucovorin in the present treatments II and III might not fit the above data definition of 'quite-high'.

From treatment II, AUC increased (1340 vs 2420 μg min ml^{-1}) significantly, but the amount of unchanged MTX excreted in the urine (X_u) appeared to be reduced ($p < 0.18$) as compared with the value from treatment I. However, from treatment III, AUC seemed to be increased ($p < 0.36$), whereas X_u decreased (18.5 vs. 10.1 μg) significantly in comparison to the value from treatment I. Therefore, the values of CL_R from treatments II and III were significantly reduced (5.84, 2.64 vs. 2.61 ml min^{-1} kg^{-1}) when compared with that from treatment I.

Chen and Chiou (1983) reported that the CL_R of unbound MTX always exceeds inulin clearance at various steady-state plasma concentrations of MTX ranging from 5.12 to 41.2 μg ml^{-1} in rabbit studies; this clearly indicated the occurrence of tubular secretion of MTX over the concentration ranges studied. Observation of tubular secretion of MTX has also been reported in human (Liegler et al., 1969) and monkey (Bourke et al., 1975). In the present study, the CL_R of unbound MTX exceeds inulin clearance from treatment I based on MTX protein binding of 56% and inulin clearance of approx. 5 ml min^{-1} kg^{-1} in rabbits (Chen and Chiou, 1983). However, the values from treatments II and III were significantly lower than that from treatment I, and were close to that of GFR. This appeared to indicate that tubular secretion of MTX was inhibited by administration of leucovorin at a dose of 20 mg kg^{-1} in the present treatments II and III. This result is consistent with a previous report (Nixon, 1979); Nixon (1979) analyzed the published (Pratt and Cooper, 1971; Goldie et al., 1972; Stoller et al., 1975; Tattersall et al., 1975) plasma and urine data of MTX in humans before and after administration of leucovorin, and concluded that there was no evidence that leucovorin increases the renal excretion of MTX, even for doses of leucovorin as high as 1000 mg. The renal tubular secretion of MTX was also reported to be blocked by salicylates and *p*-aminohippurate in humans (Liegler et al., 1969), and by probenecid in monkeys (Bourke et al., 1975). The inhibited tubular secretion of MTX from treatments II and III resulted in an increased contribution of CL_{NR} to CL; the mean values were 54.0, 65.6 and 77.5% for treatments I–III, respectively. However, the CL_{NR} values were not significantly affected by administration of leucovorin (Table 1).

Acknowledgement

This work was supported in part by a research grant from the Korea Science and Engineering Foundation, 1986–1989.

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