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Pharmacokinetics of Methotrexate after Intramuscular Injection of Methotrexate-Polylysine Conjugate in Rabbits

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Abstract□Methotrexate (MTX)-poly-L-lysine (PLL) conjugate was relatively stable in phosphate buffer of pH 7.4 and in plasma. However, liver homogenate accelerated the release of MTX from the conjugate. Pharmacokinetics and tissue distribution of MTX were compared after intramuscular injection of MTX (treatment I) and MTX-PLL conjugate (treatment II), 10 mg/kg as free MTX to rabbits. The peak concentrations of MTX in treatment II were significantly lower than those in treatment I. The amount of MTX excreted in 24-hr urine was significantly reduced in treatment II and it suggested that MTX be more metabolized in treatment II than in treatment I. The amounts of MTX remaining in each organ after 24-hr of intramuscular injection were not significantly different in both treatments.

Keywords□Methotrexate, Methotrexate-poly-L-lysine conjugate, Stability, Pharmacokinetics.

Methotrexate (MTX) has been widely used in chemotherapy of various types of neoplastic diseases[1,2]. It has systemic side effects or toxicities on G-I tract, kindey and bone marrow[3]. In order to decrease the toxicities of MTX and to provide MTX to the tumor site in a sufficient amount with a long period of time, MTX-macromolecule conjugates were synthesized and their anticancer activities were reported[4]. It was published[5] that the cellular uptake of MTX-poly-L-lysine (PLL, m.w. of 70,000 as HBr salt) conjugate was markedly enhanced in comparison with that of free MTX, and ϵ-amino groups of PLL were considered to be essential for enhancement of cellular uptake. The possible mechanism for cellular uptake of the conjugate was thought to be adsorptive pinocytosis[5]. PLL itself did not increase the cellular uptake of free MTX and MTX-PLL conjugate itself did not have anticancer activities[5]. Therefore, MTX should be released from MTX-PLL conjugate at tumor site in order to have antineoplastic activities[5].

The purpose of this note is to report the results of pharmacokinetics and tissue distribution of MTX after intramuscular (im) injection of free MTX and MTX-PLL conjugate, respectively to rabbits. Stability of the conjugate in various solutions were also investigated.

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MATERIALS AND METHODS

Materials

MTX was kindly supplied by Choong-Wae Pharm. Co. (Seoul, Korea) and PLL (m.w. of 62,500 as HBr salt) and rabbit serum albumin (RSA) were purchased from Sigma Chemical Co. (St. Louis, Mo.). The other chemicals were reagent grade and were used without further purification.

Synthesis of MTX-PLL Conjugate

The conjugate was synthesized by carbodiimide-catalyzed reaction and the contents of MTX in the conjugate were measured at 305 nm[6]. The molar ratio of MTX to PLL was 16:1, and the corresponding weight ratio was 13%.

Stability of MTX-PLL Conjugate

A weight of the conjugate equivalent to 2 mg of MTX was dissolved in 50 m*l* of phosphate buffer of pH 7.4, human plasma and rat liver homogenate, respectively. The preparation of liver homogenate was reported[7]. These mixtures were incubated in a water-bath shaker at 37°C and 50 oscillations per min (opm). Two 100 μl samples were collected with appropriate time intervals and stored in the freezer prior to HPLC assay for MTX[8].

Rabbit Studies

Five male, New Zealand White rabbits (1.5-2.1 kg) were anesthetized with ketamine (kindly supplied by Yu-Han Pharm. Co., Seoul, Korea), 50-100 mg administered intravenously (iv) via ear vein. The carotid artery was catheterized with silastic tubing (Dow Corning, Midland, Mi.) for blood sampling. Urine samples were collected using pediatric Foley catheter (Dover, Searl Medical Products, USA Ind., Dallas, Tx.) which was introduced into the urinary bladder. The animals were allowed to recover from anesthesia for 4-5 hr before the studies.

MTX, 10 mg/kg (dissolved in 0.9% NaCl strerile solution) and MTX-PLL conjugate, 10 mg/kg as MTX (freshly reconstituted with the 0.9% NaCl solution) were injected im to both legs (1.5 m*l* per each leg) of rabbits 1-3 (treatment I) and 4-5 (treatment II), respectively. The blood samples were centrifuged immediately to minimize the potential "blood strorage effect" in the plasma MTX concentration determinations[8]. Heparinized normal saline solution (10 units/m*l*), 3 m*l* was used to flush the cannula after each blood sampling to prevent blood clotting. Plasma and urine samples were stored in the freezer before HPLC assay for MTX[8]. At the end of 24-hr after im injection, the rabbits were exsanguinated. Each organ was excised, homogenized with 4 volumes of 0.01 N NaOH and centrifuged. After discarding the floating fat layer with paper towel, the supernatant was collected. The amounts of MTX remaining in each organ were measured by HPLC[8]. The area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated by trapezoidal rule-extrapolation method[10]. The renal clearance (CL_R) was estimated by dividing the total amounts of MTX excreted in urine by AUC. The mean values of clearance and half-life were estimated using harmonic mean method[9]. The data were analyzed for statistical significance ($p<0.05$) by unpaired t-test.

RESULTS AND DISCUSSION

Fig. 1 shows percentages of MTX remaining in MTX-PLL conjugate after incubation of the conjugate with various solutions at 37°C and 50 opm. The conjugate was relatively stable in phosphate buffer of pH 7.4 and in plasma; approximately 3.44 and 3.46% of MTX were released from the conjugate after 24-hr of incubation with the buffer and plasma, respectively. However, the release of MTX was accelerated in liver homogenates. For example, 52.3% of MTX were released from the conjugate after 2-hr of incubation with liver homogenates. The results are consistent with the report[5] that polylysine is very sensitive to

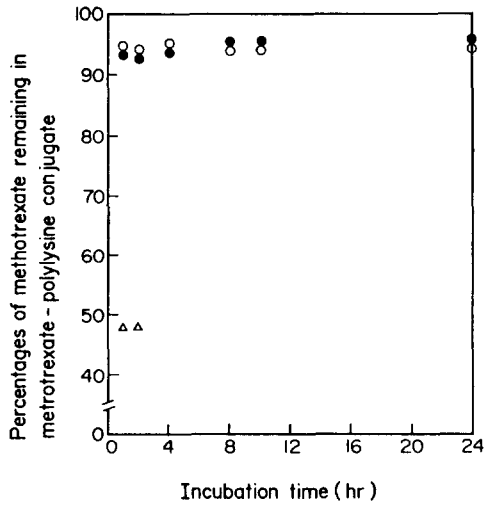

Fig. 1. Percentages of methotrexate remaining in methotrexate-polylysine conjugate after incubation of the conjugate in a water-bath shaker at 37°C and 50 oscillations per min with phosphate buffer of pH 7.4 (●), human plasma (○) and rat liver homogenate (△), respectively.

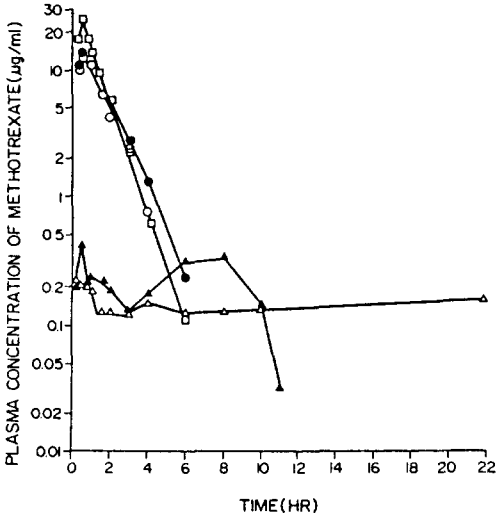

Fig. 2. Plasma concentration-time profiles of methotrexate after intramuscular injection of methotrexate, 10 mg/kg to rabbits 1 (●), 2 (○), and 3 (□), and methotrexate-polylysine conjugate, 10 mg/kg as methotrexate to rabbits 4(▲) and 5(△).

trypsin and several other proteolytic enzymes. The data after 2-hr of incubation with liver homogenates could not be obtained due to the formation of precipitation. Similar results were also obtained[11] after 6-hr of incubation of "high strength" (approximately 10% by

weight of MTX) MTX-bovine serum albumin conjugate with buffer of pH 5.0.

Fig. 2 shows plasma concentration-time profiles of MTX after im administration of MTX (treatment I) and MTX-PLL conjugate (treatment II) to rabbits. The absorption of MTX after im injection of free MTX (treatment I) was very rapid; the peak time was approximately 30 min (based on experimental data) after im injection. The peak concentrations of MTX were 14.5, 13.4 and 24.9 μg/ml for rabbits 1-3 (treatment I), respectively. The mean values of AUC and renal clearance after im administration of free MTX to rabbits 1-3 (treatment I) were 1660 ± 263 μg min/ml and 3.66 ± 1.83 ml/min/kg, respectively. MTX, 10 mg/kg, was administered iv infusion for 30-min to 4 other rabbits[12]. The mean values of AUC, total body clearance (CL), renal clearance, and nonrenal clearance were 2360 ± 536 μg.min/ml, 4.25 ± 1.05 ml/min/kg, 2.49 ± 0.969 ml/min/kg, and 1.66 ± 0.255 ml/min/kg, respectively[12]. It is well known that clearance values of MTX are dependent on plasma concentrations of MTX[13]. Therefore, the following equation was employed for the estimation of bioavailability (F), extent of absorption in the present im study[14]:

$$F = 100\ (CL_{im} \cdot AUC_{im})/DOSE_{im}$$

The F value in the present im study (treatment I) was found to be 88.3%. It indicated that MTX is "almost" completely absorbed after im administration. The rapid and almost complete absorption of MTX after im injection was also reported[15] in humans.

The plasma concentration-time profiles of MTX declined polyexponentially with apparent mean terminal half-life of 3.26 ± 0.765 hr after 30-min iv infusion of MTX, 10 mg/kg to 4 other rabbits[12]. However, MTX plasma concentrations declined monoexponentially with apparent mean terminal half-life of 0.846 ± 0.213 hr after im administration of free MTX (treatment I) in the present study (Fig. 2). The similar mean half-life, 0.988 ± 0.346 hr was also obtained from urinary excretion rate data. The plasma concentration-time profiles of MTX after im administration of MTX-PLL conjugate (treatment II) were free MTX (treatment I). For example, plasma MTX concentration rose slowly with peak concentration of concentration rose slowly with peak concentration of 0.425 and 0.226 μg/ml for rabbits 4 and 5 (treatment II), respectively and fluctuated thereafter (Fig. 2). It is of interest to note that MTX was still detected in plasma at 24-hr after im injection of MTX-PLL conjugate in rabbit 5. The plasma concentrations of MTX after 6 hr of im administration of free MTX to rabbits 1-3 (treatment I) are not shown in Fig. 2, because 8-hr plasma concentration of MTX were under detection limit based on our assay method.

The amounts of MTX excreted in 24-hr urine were significantly decreased after im injection of MTX-PLL conjugates (treatment II); the values were 10.4, 18.9, 16.9, 0.189 and 0.152 mg for rabbits 1-5, respectively. It might be due to increased metabolism of MTX after im injection of MTX-PLL conjugate (treatment II) and it was expected based on the saturable metabolism of MTX[15].

The amounts of MTX remaining at 24-hr after injection of free MTX (treatment I) and MTX-PLL conjugate (treatment II) are listenin Table I. MTX was significantly less concentrated in kidney and G-I tract when MTX-RSA conjugate was injected iv to 4 other rabbits, and it implicated that the kidney and G-I tract toxicities of MTX could be reduced by administration of MTX-RSA conjugates[12]. However, there were no significant differences in the amounts of MTX remaining in each organ when MTX-PLL conjugate was administered im (treatment II) as compared to the values when free MTX was administerd im (treatment I).

In the preliminary studies, MTX-PLL conjugate was infused iv, administered intraperitoneally or sub-

Table I. The amount of methotrexate remaining in Tissue (μg/g tissue) 24-hr After Intramuscular Adiministration of Methotrexate, 10 mg/kg and Methotrexate-Polylysine conjugate, 10 mg/kg as MTX to Rabbits 1-3 (treatment I) and 4-5 (treament II), Respecitively

	Treatment I			Treatment II	
Rabbit	1	2	3	4	5
Liver	0.5691	0.4967	0.6105	0.3929	0.7140
Lung	–	0.1917	0.2481	–	0.3045
Hart	0.2484	0.2484	0.3415	0.3295	0.2794
Stomach	–	–	0.2515	–	0.1770
Kidney	0.3400	0.8665	0.9433	0.4148	0.3620
Brain	0.2354	0.2448	0.3202	–	0.2166
Spleen	–	–	–	0.1446	–
Bile	–	–	–	–	–
Muscle	0.5433	0.4139	0.4398	0.4892	0.3622
Fat	–	–	–	–	–
Mesentary	–	–	–	–	–
Small intestine	0.2805	0.7668	0.4170	0.4418	0.5785
Large intestine	0.1848	–	0.1751	–	0.1751
Testis	–	0.1904	0.1999	–	0.1999

–: Not detected

cutaneously to rabbits. Unfortunately, all the rabbits died immediately. It might be due to the toxicities of PLL itself as reported by Ryser and Shen[5]. The toxicities of MTX-PLL conjugate should be considered if the conjugates are disigned to treat neoplastic diseases.

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