Clearance Studies of Methotrexate in Dogs after Multiple-Rate Infusion

Chung Y. Lui, Myung G. Lee, and Win L. Chiou

ABSTRACT

Plasma, renal, and nonrenal clearances of methotrexate as well as their interrelationship were studied in five conditioned male beagle-mongrel dogs using the multiple-rate infusion method. Steady-state plasma methotrexate concentrations of 1, 20, and 100 μg/ml were targeted for by i.v. bolus doses followed by i.v. infusions. An isotonic solution of sodium bicarbonate or ammonium chloride was simultaneously infused to study the effect of acid-base imbalance on the clearances. NaCl solution (0.9%) infusion served as a control. Plasma and urine concentrations of methotrexate were quantitated by a sensitive high-performance liquid chromatographic method. Distortion of body acid-base balance did not significantly change the clearances of methotrexate. The results showed that the plasma clearance (4.02 to 4.68 ml/min/kg) of methotrexate was relatively constant over the concentration range studied. The renal and nonrenal clearances, on the other hand, were concentration dependent. As the plasma methotrexate concentration increased from 1 to 20 or 100 μg/ml, renal clearance decreased from 3.60 to 4.28 ml/min/kg to 2.62 to 2.73 ml/min/kg, and nonrenal clearance increased from 0.35 to 0.42 ml/min/kg to 1.38 to 1.74 ml/min/kg. Concentration-dependent renal clearance may be due to saturation of the process involving active tubular secretion of methotrexate.

INTRODUCTION

The pharmacokinetics of methotrexate have been under extensive investigation for more than a decade. However, there are markedly different results reported regarding its renal excretion in humans with apparent normal renal function. For example, renal clearances in low doses (<20 mg/kg) ranged from 18 ml/min (16) to 179 ml/min (7), whereas those in high doses varied from 20 to 50 ml/min (25) to 104 ml/min (13). These variations may arise partly from the real difference among individuals examined and/or from the nonspecific assays used (3, 11). In addition, 7-hydroxy methotrexate, the major metabolite of methotrexate (2, 6, 15, 19), may interfere with the renal excretion of methotrexate, because 7-hydroxy methotrexate has been shown to compete with methotrexate for transport into tumor cells (15). Therefore, it seems that the renal clearance of methotrexate and consequently the interrelationship between its renal and nonrenal elimination may not be as well "defined" as it would be. Dogs were selected as the animal model because they do not metabolize methotrexate. Although Huang et al. (12) have studied the renal excretion mechanism of methotrexate in dogs, they did not report the interrelationship between its renal and nonrenal clearances.

In high-dose methotrexate therapy, a fairly large amount of sodium bicarbonate is usually prescribed to patients to maintain the urine pH above 7.0 (2, 13, 22). This practice, however, may distort the acid-base balance of the patients (20). Therefore, it may be of interest to study the effect of change in plasma pH on the clearance of methotrexate. Again, the dog was used as an animal model for this investigation.

The multiple steady-state infusion approach was used in the present study. This may avoid the potential complication due to arterial-venous differences following single i.v. administration (4, 14) and also provide simplicity in clearance calculations.

MATERIALS AND METHODS

Animals and Chemicals. Five conditioned male unanesthetized beagle-mongrel (hybrid of mongrel and beagle) dogs (7.7 to 14 kg) were used. Urine collection was made from an indwelling polypropylene urinary catheter (No. 5 French catheter, 56 cm; Sovereign, St. Louis, MO), and infusion of solution or blood sampling was from an i.v. cannula (5 cm, 22 g; Sovereign) placed into the cephalic vein of the dogs. Cross-over experiments were carried out at 2-week intervals.

Methotrexate (10 and 50 mg/ml) was kindly supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, NY, and the National Cancer Institute, Bethesda, MD. Inulin and sodium chloride injection (United States Pharmacopoeia) was generously donated by American Critical Care, Division of American Hospital Supply Corp., McGaw Park, IL. Sodium bicarbonate (5%), sodium chloride (0.9%), and ammonium chloride (2.14%) injection (United States Pharmacopoeia) were purchased from American McGaw, Division of American Hospital Supply Corp., Irvine, CA. Water for injection (United States Pharmacopoeia) was used to prepare the isotonic solution for sodium bicarbonate (1.5%) and ammonium chloride (1.0%).

Methods. The dogs were fasted for 18 to 24 h prior to the experiments and restrained by means of a dog sling (Alice King Chatham Medical Arts, Los Angeles, CA). Steady-state plasma methotrexate concentrations of 1, 20, and 100 μg/ml were targeted for by i.v. bolus doses of approximately 0.5, 10.0, and 50.0 mg/kg followed by i.v. infusions of approximately 4.5, 90.0, and 450.0 μg/ml/min, respectively. A 10% inulin solution was simultaneously infused for renal function monitoring. The dogs were hydrated by infusion, with 100 to 300 ml of one of the isotonic solutions (12 ml/min for sodium bicarbonate or 2.2 ml/min for ammonium chloride; higher rates of ammonium chloride were found to cause vomiting) before methotrexate and inulin were administered. Thereafter, the isotonic solution was infused at a rate of 2.2 ml/min throughout the entire experiment to distort the acid-base balance (ammonium chloride and sodium bicarbonate treatments only) and facilitate urine collection. All solutions were infused by means of a Harvard Apparatus Compact infusion pump (Model 975; Harvard Apparatus Co., Millis, MA). For the control study, the 3 steady-state plasma methotrexate concentrations were attained within an experiment in a stepwise fashion in the same day. For the ammonium chloride or sodium bicarbonate study, only the 2 higher concentrations were similarly attained within the experiment; the plasma concentration of 1 μg/ml for these 2 treatments was achieved in separate experiments. The maintenance infusion rate in these separate experiments was modified because the purpose was also to induce

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2 Abstracted from a dissertation submitted in 1984 by C. Y. Lui to the Graduate College, University of Illinois at Chicago, in partial fulfillment of Ph.D. degree requirements.
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changes in urine flow rate and pH for a different study.

Measurements of renal and plasma clearances were started 60 min after the beginning of i.v. infusion of methotrexate and inulin (adequate to reach steady state), and ended after 4 clearance periods (30 min each) had been completed for each one of the steady-state concentrations studied. However, 8 to 10 clearances were measured for dogs infused with bicarbonate or ammonium chloride solution at the targeted concentration of 1 μg/ml. At the end of each interval, the bladder was flushed 3 times with 20 ml of sterile water for irrigation (United States Pharmacopoeia) (Travenol Laboratories Inc., Deerfield, IL) and 20 to 40 ml of air to ensure complete recovery of urine. One ml of blood was drawn at the beginning and end of each urine collection period. Plasma was immediately separated to minimize the potential “blood storage effect” (17, 18). Both plasma and urine samples were frozen prior to analysis.

Venous blood pH values were measured before and during methotrexate and inulin administration. Three ml of venous blood was withdrawn, and only the middle 1-ml portion was used for pH determination at 25 °C. The blood pH was measured anaerobically by covering the blood with about 1 ml of mineral oil.

Analytical Procedure. Methotrexate was quantitated by modification of a high-performance liquid chromatographic method developed in our laboratory (3). The mobile phase was 1 volume of acetonitrile and 9 volumes of 0.035 M monobasic ammonium phosphate acidified with 85% phosphoric acid (1 ml/liter). The assay limit was 0.1 μg/ml for plasma and urine. The coefficients of variation for intra- and interday assays were 1.04 and 3.32%, respectively.

Inulin was assayed according to the colorimetric method of Higashi and Peters (10). The procedure was scaled down to analyze 100 μl of plasma or urine.

Pharmacokinetic and Statistical Analysis. The plasma clearance (CL) was calculated by:

\[ CL = \frac{R_0}{C_w} \]

where \( R_0 \) is the infusion rate and \( C_w \) is the steady-state plasma concentration. Renal clearance (CLr) was calculated by the timed-interval method:

\[ CL_r = \frac{U_{t_1-t_2}}{AUC_{t_1-t_2}} \]

where \( U_{t_1-t_2} \) is the amount of drug excreted unchanged in the urine during the time interval \( t_1 - t_2 \), and \( AUC_{t_1-t_2} \) is the area under the plasma concentration time curve between \( t_1 - t_2 \). This area was calculated by the linear trapezoidal rule. Nonrenal clearance (CLn) was estimated by the difference between plasma and renal clearances.

The data were analyzed for statistical significance (\( P < 0.05 \)) by analysis of variance with dogs as a block using a GLM procedure (24).

RESULTS AND DISCUSSION

Table 1 summarizes the pharmacokinetic parameters of the multiple infusion studies in 5 dogs. As noted in the previous study (4) that the renal clearance of methotrexate at a plasma concentration of 1 μg/ml was independent of changes in urine flow and pH during the administration of sodium bicarbonate or ammonium chloride solution, the clearances obtained were listed along with those measured under constant urine flow rate. Representative data for plasma levels attained in 3 dogs during each infusion rate of methotrexate are depicted in Chart 1. As shown in Chart 1, steady-state levels of methotrexate were in all practicality achieved in this study by an i.v. infusion preceded by an appropriate priming dose.

As shown in Table 1, the steady-state plasma methotrexate concentrations achieved in all 3 treatments were, in general, in good agreement with those predicted. For all treatments, nonlinear kinetics of methotrexate were observed in all but one parameter monitored, the plasma clearance. With increased infusion rate, there was a proportional increase in the steady-state plasma levels, indicating dose-independent elimination of the drug. This result agrees with the reported concentration-independent elimination of methotrexate in patients receiving short-term (4 to 6 h) infusions (2, 13, 27). However, dose-dependent plasma clearance of methotrexate has been demonstrated in rabbits (5) and some other studies in humans (6, 16, 22). The reason for these apparently conflicting results is not known at the present time.

In Table 1, an increase in steady-state plasma methotrexate concentration from 1 to 20 or 100 μg/ml in each treatment resulted in a statistically significant increase in CLn, as well as in a statistically significant decrease in CLr. For all treatments, the nonrenal elimination of methotrexate contributed about 8 to 11% and 33 to 39% of the total elimination at the lower (1 μg/ml) and higher concentrations (20 and 100 μg/ml), respectively. The observed 8 to 11% contribution by the nonrenal route was similarly reported by Bischoff et al. (1) and Henderson et al. (9). It is difficult, however, to compare the nonrenal clearance of the present study with that of rabbits (5) and humans (2, 6), because these 2 species metabolize methotrexate and hence their nonrenal clearances were measurements of both metabolic and presumably biliary excretion clearances (1, 9, 25, 26). Again, there was no significant difference in renal and nonrenal clearances among the treatments.

The compensatory clearances (renal and nonrenal) of methotrexate in the present study appeared to be partly related to the saturation of the renal excretion mechanism as judged from the fairly constant renal clearance at plasma concentrations of approximately 20 and 100 μg/ml (Table 1). As a result, the clearance of methotrexate by the other route, presumably biliary, may become more important at relatively higher concentrations. Biliary excretion of methotrexate in dogs has been documented (1, 9). If the nonrenal elimination of methotrexate in the present study is biliary clearance, the observed concentration-dependent nonrenal clearance may be a result of saturation of the process involving biliary reabsorption of methotrexate, because its dependence on steady-state concentration is similar to renal excretion of drugs with carrier-mediated reabsorption (23), i.e., a rise in steady-state concentration will result in an increase in renal clearance. Furthermore, reabsorption of organic compounds such as glucose (21) and phenolphthalein glucuronide (8) by the biliary tract has been reported.

The compensatory phenomenon observed in the present study may be different from that observed in the earlier rat study (26). It was reported (26) that ligation of the bile duct resulted in a 1.7- to 2.5-fold increase in the percentage of methotrexate excreted into the urine, while nephrectomy increased the percentage of biliary excretion 1.2- to 1.7-fold. However, this percentage increase in elimination may not reflect the “real” compensatory phenomenon because, when elimination is linear, clearance of the drug by either route will remain relatively unchanged, although the percentage of dose eliminated by either route will change markedly. On the other hand, the nonrenal clearance of methotrexate observed in the present study did increase from 0.35 to 0.40 ml/min/kg to 1.38 to 1.74 ml/min/kg when the saturation of the active transport process in the renal
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Table 1
Summary of methotrexate clearances from multiple-infusion studies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Steady-state concentration (µg/ml)</th>
<th>Infusion rate (µg/kg/min)</th>
<th>Plasma clearance (mL/min/kg)</th>
<th>Renal clearance (mL/min/kg)</th>
<th>Nonrenal clearance (mL/min/kg)</th>
<th>Renal clearance (mL/min/kg)</th>
<th>Inulin clearance (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium chloride infusion</td>
<td>0.96 ± 0.34^b</td>
<td>4.39 ± 1.33</td>
<td>4.68 ± 0.51</td>
<td>4.28 ± 0.43</td>
<td>0.40 ± 0.17</td>
<td>0.92 ± 0.03</td>
<td>5.22 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>20.2 ± 0.64</td>
<td>86.3 ± 2.74</td>
<td>3.31 ± 0.17</td>
<td>2.62 ± 0.18</td>
<td>1.69 ± 0.30</td>
<td>0.61 ± 0.06</td>
<td>4.47 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>98.2 ± 11.9</td>
<td>417 ± 35</td>
<td>4.28 ± 0.28</td>
<td>2.65 ± 0.58</td>
<td>1.63 ± 0.39</td>
<td>0.62 ± 0.10</td>
<td>4.54 ± 0.94</td>
</tr>
<tr>
<td>Sodium chloride infusion</td>
<td>1.10 ± 0.22</td>
<td>4.75 ± 1.04</td>
<td>4.02 ± 0.54</td>
<td>3.60 ± 0.51</td>
<td>0.42 ± 0.33</td>
<td>0.89 ± 0.06</td>
<td>4.23 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>20.4 ± 1.77</td>
<td>90.4 ± 6.70</td>
<td>4.47 ± 0.14</td>
<td>2.72 ± 0.32</td>
<td>1.74 ± 0.38</td>
<td>0.64 ± 0.09</td>
<td>4.33 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>102 ± 9.50</td>
<td>410 ± 34.3</td>
<td>4.07 ± 0.35</td>
<td>2.69 ± 0.17</td>
<td>1.38 ± 0.48</td>
<td>0.67 ± 0.09</td>
<td>4.08 ± 0.31</td>
</tr>
<tr>
<td>Sodium bicarbonate infusion</td>
<td>1.15 ± 0.34</td>
<td>4.91 ± 1.37</td>
<td>4.29 ± 0.19</td>
<td>3.94 ± 0.10</td>
<td>0.35 ± 0.14</td>
<td>0.91 ± 0.03</td>
<td>4.92 ± 0.94</td>
</tr>
<tr>
<td></td>
<td>19.9 ± 1.88</td>
<td>86.4 ± 7.75</td>
<td>4.39 ± 0.17</td>
<td>2.73 ± 0.35</td>
<td>1.65 ± 0.20</td>
<td>0.63 ± 0.06</td>
<td>4.31 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>100 ± 9.50</td>
<td>428 ± 30.1</td>
<td>4.29 ± 0.21</td>
<td>2.72 ± 0.10</td>
<td>1.57 ± 0.19</td>
<td>0.63 ± 0.03</td>
<td>4.30 ± 0.43</td>
</tr>
</tbody>
</table>

Statistically significant (P < 0.05) difference was observed only at 1 µg/ml and 20 µg/ml and 1 and 100 µg/ml were observed in each treatment.

Chart 1. Representative plasma level (µg/mL) as a function of infusion time (min) attained during each infusion rate of methotrexate. A, plasma concentration of approximately 1 µg/mL; B, 20 µg/mL; C, 100 µg/mL. •, Dog 5, treated with ammonium chloride; ●, Dog 3, infused with 0.9% NaCl solution; ▲, Dog 4, treated with sodium bicarbonate.

Table 2
"Unbound" methotrexate/inulin renal clearance ratio at 3 different steady-state concentrations and treatments from multiple-infusion studies

<table>
<thead>
<tr>
<th>Steady-state concentration (µg/ml)</th>
<th>Ammonium chloride infusion</th>
<th>Sodium chloride infusion</th>
<th>Sodium bicarbonate infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µg/ml</td>
<td>1.30</td>
<td>1.35</td>
<td>1.27</td>
</tr>
<tr>
<td>20 µg/ml</td>
<td>0.93</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>0.93</td>
<td>1.04</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Statistically significant (P < 0.05) difference was observed only at 1 µg/ml in each treatment.

Distortion of the body acid-base balance by the infusion of an isotonic solution of sodium bicarbonate or ammonium chloride, however, did not appear to modify the clearances of methotrexate (Table 1). The measured venous blood pHs before and during infusion of ammonium chloride solution were 7.53 ± 0.04 (SD) and 7.23 ± 0.11, respectively. These blood pHs were determined in experiments in which steady-state methotrexate concentrations were 20 and 100 µg/mL. Unfortunately, blood pH of dogs given infusions of sodium bicarbonate solution was not measured. However, when blood pHs were measured in experiments similar to the present study, the pHs were found to be 7.51 ± 0.04 for the control and 7.67 ± 0.08 during infusion of sodium bicarbonate solution.

Table 2 shows the mean values of the ratio of "unbound" methotrexate to inulin renal clearance in 5 dogs at 3 different steady-state concentrations after the 3 different treatments. A plasma protein binding of 36.9% determined in a previous study was used to estimate these ratios. As listed in Table 2, at steady-state concentrations of 20 and 100 µg/mL, the "unbound" renal clearance ratio approached unity for all treatments. This may indicate that the renal clearance of methotrexate is practically due to glomerular filtration. At a steady-state concentration of 1 µg/mL, however, renal clearance is a combination of active secretion and glomerular filtration. Passive reabsorption of methotrexate did not appear to play a significant role because, as noted in another study, renal clearance of methotrexate was insensitive to urine flow rate and pH change. Although the "unbound" ratios at this concentration were moderately greater than one, the ability of the renal tubules of these dogs to secrete the drug was relatively weak when a comparison was made with rabbits (5) and monkeys (12). Again, no significant treatment difference was observed.

It is of interest to note that, upon repeated administration of methotrexate to dogs, the clearances of methotrexate were very similar among the treatments. This shows that the overall disposition of methotrexate in dogs did not change over time, although different solutions of electrolyte were infused.

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