Urinary pH and Urine Flow Independent Renal Clearance of Methotrexate in Dogs

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The effects of urine flow and pH on methotrexate renal clearance were studied in seven conditioned male Beagle-Mongrel dogs. Steady-state plasma methotrexate and inulin concentrations were achieved by i.v. infusions preceded by i.v. bolus doses. Plasma and urine concentrations of methotrexate were quantitated by a sensitive high-performance liquid chromatographic assay, while those of inulin were measured by a colorimetric method. Since plasma protein binding of methotrexate was pH and concentration independent, methotrexate/inulin renal clearance without correcting for plasma binding was used for most of the data analyses. The results showed that the renal clearance ratios at the plasma methotrexate levels (approximately 0.1, 1.0, 20.0, and 100 µg/ml) studied remained relatively constant when urine pH (differences of up to about 2.5 units) and flow rate (differences of up to approximately 30 times) were changed. This indicated that renal reabsorption of methotrexate in these dogs was negligible. However, concentration-dependent renal clearance was observed. The mean renal clearances were 3.84, 3.94, 2.73, and 2.72 ml/min/kg at plasma concentrations of about 0.1, 1.0, 20.0, and 100.0 µg/ml, respectively, when urine was alkalized by sodium bicarbonate. The corresponding clearances were 4.02, 4.28, 2.62, and 2.65 ml/min/kg when urine was acidified by ammonium chloride. These showed the existence of saturable tubular secretion of methotrexate. No 7-hydroxy-methotrexate, a metabolite found in other species, was detected in the urine and plasma of the dogs.

KEY WORDS: methotrexate renal clearance; urine pH and flow independent; plasma concentration dependent.

INTRODUCTION

High dose methotrexate therapy in combination with citrovorum factor rescue has been widely used in the treatment of various malignant neoplasms.

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Due to the low aqueous solubility of methotrexate and its metabolite, 7-hydroxy-methotrexate, in acidic pH, forced diuresis and alkalization of urine are routinely prescribed in patients receiving high-dose therapy in order to avoid crystalluria and improve renal excretion (2-5). There are conflicting results, however, in the use of this regimen. Some have reported that hydration (6,7) and alkalization of urine (8) were not much help in increasing methotrexate renal elimination, but did decrease incidents of acute nephrotoxicity and subsequent myelotoxicity (9). Sand and Jacobsen (10) demonstrated urinary pH but not urine flow dependent renal clearance based on venous data after a single dose. How kidneys handle methotrexate in man is still not yet fully understood. Both passive (5) and active (11,12) reabsorption in the renal tubules have been proposed. Others have excluded nonionic diffusion as a significant renal mechanism because of the negligible chloroform-aqueous partition coefficient of methotrexate (13).

The purpose of this paper is to investigate systematically in dogs the effects of altering pH and flow rate of urine on the renal clearance of methotrexate. Inulin renal clearance and steady-state infusion methods were used to minimize the potential drawbacks in renal clearance study (14).

**MATERIALS AND METHODS**

**Animals and Materials**

A total of seven conditioned male Beagle-Mongrel hybrid dogs (7.7-14 kg) were used. For each study 4-5 dogs were involved and the experiments were conducted in a cross-over fashion with alkalization being carried out first. The dogs were allowed to rest for 2 weeks in between experiments. The dogs were deprived of water in some studies for the purpose of inducing initial low urine flow and fasted for 18-24 hr prior to the experiments. They were conscious throughout the entire experimental procedure and no anesthetic agents or tranquilizing drugs were administered at anytime. The dogs were restrained by means of a dog sling (Alice King Chatham Medical Arts, Los Angeles, California). An indwelling polypropylene urinary catheter (5 Fr., 22 in., Sovereign, St. Louis, Missouri) inserted into the urinary bladder prior to each study was used to collect urine. An intravenous cannula (2 in., 22 g., Sovereign) was placed into the cephalic vein of each foreleg for infusion of blood sampling.

Methotrexate solutions (in 0.9% sodium chloride injection, U.S.P.) of approximately 10 and 50 mg/ml were prepared. The drug was generously supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York and the National Cancer Institute, Bethesda, Maryland. Inulin and sodium chloride injection, U.S.P. was generously donated by American Critical Care, Division of American Hospital Supply Corpor-