Drug Absorption VIII: Kinetics of GI Absorption of Methotrexate

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Abstract □ The absorption of methotrexate from the lumen of the rat small intestine, *in situ*, was found to obey Michaelis–Menten kinetics. The values of V_{max} and K_m for the absorption process were 4.78×10^{-7} M/min and 1.49×10^{-5} M, respectively.

Keyphrases □ GI absorption—methotrexate from rat small intestine *in situ* □ Absorption, GI—methotrexate from rat small intestine *in situ* □ Methotrexate—GI absorption from rat small intestine *in situ* □ Antineoplastic agents—methotrexate, GI absorption from rat small intestine *in situ*

Previous papers in this series dealt with passive drug absorption from the rat GI lumen *in situ*. In some cases, the unionized drug form was the only species absorbed (1); in other cases, significant absorption of drug ions was observed (2). The blood perfusion rate of the GI membrane affected the passive absorption rate (3), but other physiological factors and the active pumping of a drug across the membrane have not been studied.

Methotrexate uptake by L-1210 mouse leukemia cells (4) and by other *in vivo* tissue and organ systems (5) was described in terms of Michaelis–Menten enzyme kinetics. It also was suggested that the GI absorption of methotrexate in mice is a saturable process (6), but the absorption kinetics have not been studied directly.

This study determined if methotrexate uptake from the lumen of the rat small intestine *in situ* obeys Michaelis–Menten enzyme kinetics and evaluated the kinetic uptake parameters.

EXPERIMENTAL

Male Sprague–Dawley rats[1], 200–250 g, were kept under observation for 7–10 days. Food was withdrawn 16–20 hr prior to the experiments, but water was allowed *ad libitum*. The rats were weighed and anesthetized with injections of ketamine hydrochloride[2], 60 mg/kg im, and pentobarbital sodium[3], 21 mg/kg ip. When additional doses of anesthetic were needed during an experiment, one-third of the initial doses of both agents were administered by the same routes.

Isolation and cannulation of the small intestine were carried out as previously described (1). The intestine was washed with normal saline. The drug solution was instilled, and 0.1-ml samples were collected every 5 min for 30 min.

Methotrexate[4] was prepared as a 10^{-4} M stock solution in 0.2% sodium bicarbonate and stored at 4° in a flask wrapped in aluminum foil. Although such solutions are stable for over 1 month (7), fresh stock solutions were prepared weekly. A stock solution of 3′,5′,9(*n*)-^{3}H-methotrexate sodium salt[5] in water was prepared such that 100 μl contained 0.1 μCi of radioactivity (2×10^{-11} mole of methotrexate). The stock solution containing radioactive methotrexate was frozen and stored in an amber multidose container under nitrogen.

Solutions for instillation into the intestine were prepared by diluting the stock solution of methotrexate to the desired concentration with normal saline and adding 100 μl of the radiolabeled stock solution. Thus, the radiolabeled methotrexate did not add significantly to the total concentrations of the solutions.

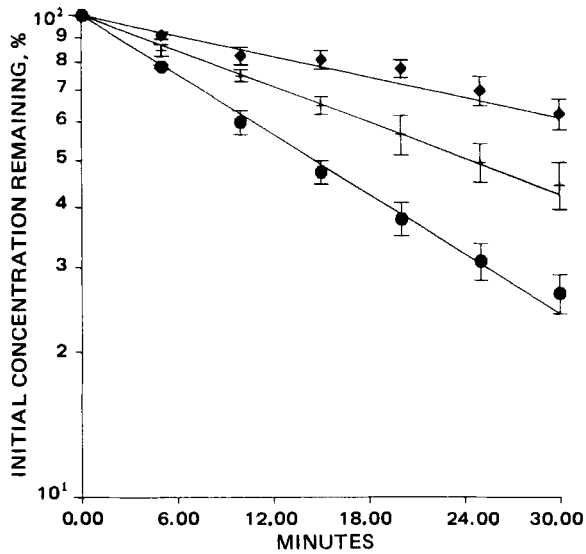

Figure 1—*Semilog plot of percent of initial concentration of methotrexate remaining* versus *time in the lumen of the* in situ *rat small intestine. The points are observed values (±SD). Initial concentrations of methotrexate were 1×10^{-5} M (◆), 1×10^{-6} M (+), and 1×10^{-8} M (●).*

The disappearance of methotrexate from the gut lumen was followed by removing 0.1-ml samples at 5-min intervals, diluting the samples in 15 ml of a scintillation cocktail [2,5-diphenyloxazole[6] (4 g), 1,4-bis[2-(5-phenyloxazolyl)]benzene[6] (0.1 g), octoxynol[6] (360 g), and toluene[6] (*q.s.* to 1000 ml)], and counting on a scintillation counter[7]. The counting efficiency was 26%. To confirm that the scintillation counting reflected only absorption of unchanged methotrexate, several samples at several concentrations from different animals were subjected to TLC analysis. Samples removed at 5, 15, and 30 min were spotted on TLC plates[8] and developed with acetic acid–acetone–methanol–benzene (5:5:20:70). After development, the chromatogram was scanned with a radiochromatogram scanner[9]. Each chromatogram contained only one spot. This spot had an R_f value identical to that of unchanged methotrexate.

RESULTS AND DISCUSSION

Semilogarithmic plots of percent of initial concentration of methotrexate remaining unabsorbed *versus* time are shown in Fig. 1. The apparent half-life of the absorption process increased from 15 min with an initial methotrexate concentration of 10^{-8} M to 45 min with an initial methotrexate concentration of 10^{-5} M. This decreasing absorption rate with increasing initial concentration of methotrexate indicated that absorption was nonlinear.

Figure 2 shows a plot of the fraction of administered dose absorbed from the gut lumen after 30 min *versus* the initial methotrexate concentration for a wide range of initial methotrexate concentrations. The

[1] Charles Rivers, Wilmington, MA 01887.
[2] Ketaject, Bristol Laboratories, Syracuse, NY 13201.
[3] Nembutal Sodium solution, Abbott Laboratories, North Chicago, IL 60064.
[4] Lederle Laboratories, Division of American Cyanamid Co., Pearl River, NY 10965.
[5] Amersham/Searle Corp., Arlington Heights, IL 60005.
[6] Scintillation grade, Research Products International Corp., Elk Grove Village, IL 60007.
[7] Packard Tri-Carb model 3375, Packard Instrument Co., Downers Grove, Ill.
[8] Silica gel G, EM Reagents Division, Brinkmann Instruments, Westbury, NY 11590.
[9] Model 7201, Packard Instrument Co., Downers Grove, Ill.

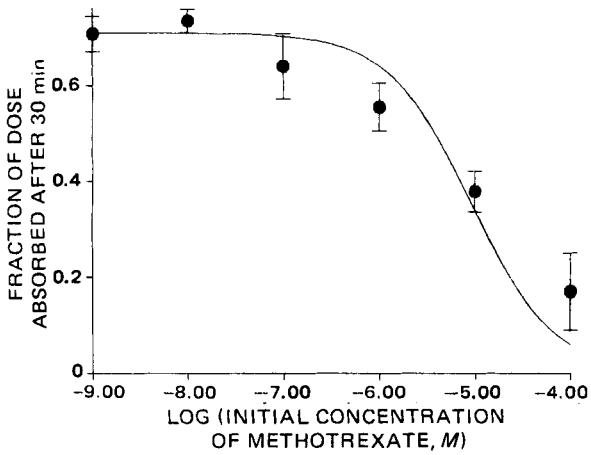

Figure 2—*Plot of fraction of dose absorbed after 30 min* versus *log (initial methotrexate concentration). The points were experimentally observed* (±SD), *and the line was calculated using Eq. 1 with* $K_m = 1.07 \times 10^{-5}$ M *and* $F_{max} = 0.71$.

value of F_{max} was used because the initial velocity could not be measured accurately at higher concentrations; therefore, this type of plot, obtained over a wide range of concentrations, gave an initial estimate of K_m for further investigations. At initial methotrexate concentrations greater than 10^{-7} M, there was a marked reduction in the fraction of the dose absorbed in 30 min, suggesting that the absorption process became saturated above this initial concentration.

The data shown in Fig. 2 were fitted, using the NONLIN program (8), to the following Michaelis–Menten equation (9):

$$F = (F_{max}K_m)/(K_m + X) \qquad \text{(Eq. 1)}$$

where F is the fraction of the administered dose absorbed after 30 min, F_{max} is the maximum fraction absorbed in 30 min, K_m is the Michaelis constant, and X is the initial concentration of methotrexate instilled in the gut lumen. The NONLIN calculated value of K_m was $1.07 \pm 0.35 \times 10^{-5}$ M, and the calculated value of F_{max} was 0.71 ± 0.03. These values and Eq. 1 were used to generate the lines in Fig. 2.

To obtain more accurate estimates of the Michaelis–Menten parameters, the absorption kinetics were further investigated using a narrow range of initial methotrexate concentrations near the calculated K_m value

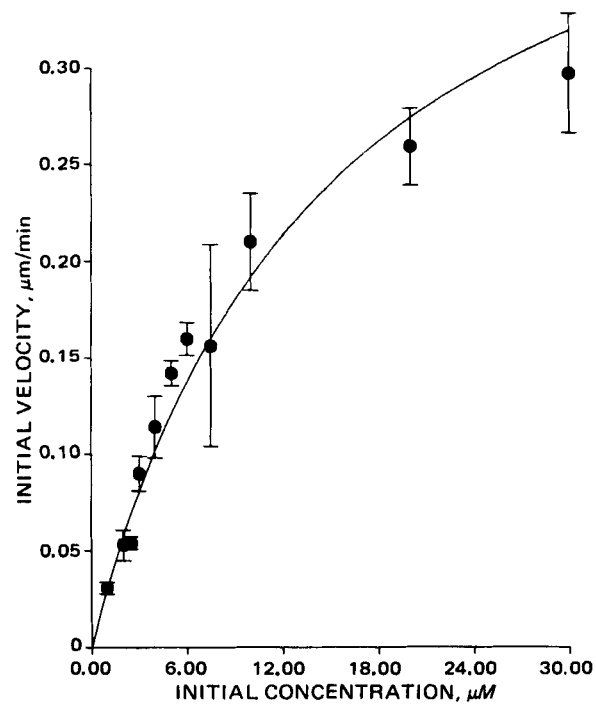

Figure 3—*Plot of initial velocity* versus *initial concentration. The points were experimentally observed* (±SD), *and the line was calculated using Eq. 2 with* $K_m = 1.49 \times 10^{-5}$ M *and* $V_{max} = 4.78 \times 10^{-7}$ M.

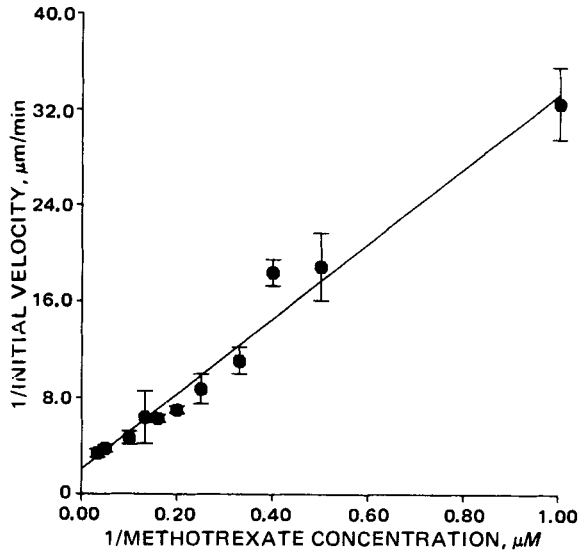

Figure 4—*Lineweaver–Burk-type plot of the data shown in Fig. 3. The points* (±SD) *were experimentally determined.*

of 1.07×10^{-5} M. Figure 3 shows a plot of the initial velocity of absorption *versus* the initial methotrexate concentration from 1×10^{-6} to 3×10^{-5} M. These data were fitted, using the NONLIN program, to the following Michaelis–Menten equation (9):

$$V = (V_{max}X)/(K_m + X) \qquad \text{(Eq. 2)}$$

where V is the observed initial velocity, V_{max} is the maximum initial velocity, and K_m and X are as defined in Eq. 1. During the fitting procedure, the data were weighted by the reciprocal of the variance. The variance of each point was calculated following linear regression of log (observed standard deviation)^2 *versus* log V (10). The calculated values of V_{max} and K_m were $4.78 \pm 0.75 \times 10^{-7}$ M/min and $1.49 \pm 0.32 \times 10^{-5}$ M. Figure 4 is a Lineweaver–Burk-type plot (11) of the data shown in Fig. 3. The solid lines in Figs. 3 and 4 were generated using Eq. 2 with $V_{max} = 4.78 \times 10^{-7}$ M/min and $K_m = 1.49 \times 10^{-5}$ M.

Absorption was saturable at a relatively low methotrexate concentration, suggesting that saturation could occur following therapeutic administration of methotrexate. This result is consistent with reports that higher methotrexate doses are incompletely absorbed in humans (12).

This study also demonstrates the utility of the *in situ* rat small intestine preparation for investigating active absorption processes.

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