Competitive Inhibition between Folic Acid and Methotrexate for Transport Carrier in the Rat Small Intestine

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Abstract □ Folic acid absorption from the lumen of the rat small intestine *in situ* obeyed Michaelis–Menten kinetics. The values of V_{max} and K_m for absorption were 4.63×10^{-7} M/min and 1.21×10^{-6} M, respectively. Folic acid and methotrexate were mutual competitive inhibitors of absorption. Their K_i values were 1.28×10^{-6} and 1.9×10^{-5} M, respectively.

Keyphrases □ Folic acid—absorption, rat small intestine, competitive inhibition by methotrexate □ Methotrexate—absorption, rat small intestine, competitive inhibition by methotrexate □ Antineoplastic agents—methotrexate, absorption, rat small intestine, competitive inhibition by folic acid □ Intestinal absorption—folic acid, methotrexate, competitive inhibition, rats

Methotrexate absorption from the lumen of the rat small intestine *in situ* is a saturable process which obeys Michaelis–Menten kinetics (1). The values of V_{max} and K_m for this process were found to be 4.78×10^{-7} M/min and 1.49×10^{-5} M, respectively. Transport of methotrexate into various tumor cells and normal isolated murine intestinal cells *in vitro* has also been shown to be saturable (2–4). Furthermore, it was reported that folic acid uptake from the rat jejunum is a saturable process (5, 6). Since folic acid and methotrexate act on the same liver enzyme system (7), it was of interest to determine if they are transported by the same transport carrier in the small intestine.

The purposes of this study were to determine the Michaelis–Menten constants, V_{max} and K_m, for folic acid uptake from the rat small intestine *in situ* and to determine if folic acid and methotrexate are inhibitors of one another's transport in the rat small intestine.

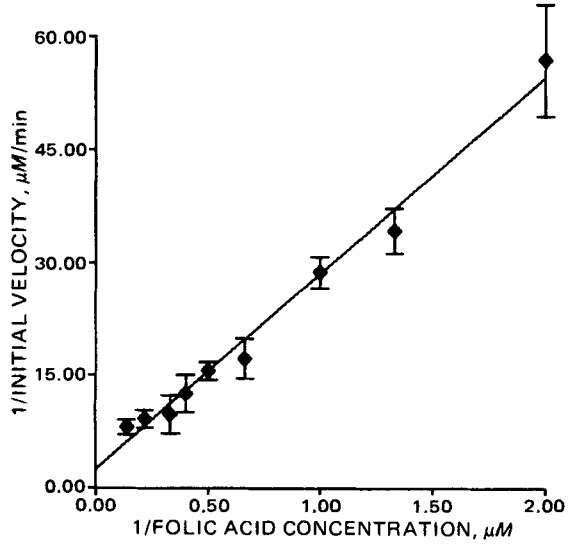

Figure 1—*Lineweaver–Burk plot (10) of absorption velocity* versus *folic acid concentration in the lumen of the rat small intestine* in situ *(mean of six animals ± SD).*

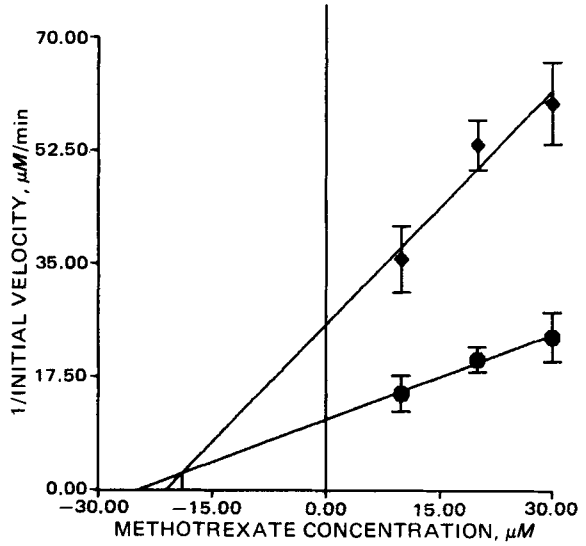

Figure 2—*Reciprocal of initial folic acid absorption velocity at 1×10^{-6} (●) and 2.5×10^{-6} (♦) M folic acid in the presence of 1×10^{-5}, 2×10^{-5}, and 3×10^{-5} M methotrexate (mean of four animals ± SD).*

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 ketamine hydrochloride[2], 60 mg/kg im, and pentobarbital sodium[3], 21 mg/kg ip, as described previously (1).

Isolation and cannulation of the small intestine were carried out as described previously (8). The intestine was washed with normal saline. The drug solution was instilled, and 0.1-ml samples were collected every 5 min for 20 min.

Folic acid[4] and methotrexate[5] were prepared as 10^{-4} M stock solutions in 0.2% sodium bicarbonate and stored at 4° in flasks wrapped in aluminum foil. Although such solutions are stable for over 1 month (9), fresh stock solutions were prepared weekly. Stock solutions of 3′,5′,9(n)-^{3}H-methotrexate sodium salt[6] and of 2-^{14}C-folic acid potassium salt[6] in water were prepared such that 100 μl contained 0.1 μCi of radioactivity (2×10^{-11} M with respect to each drug). The radioactive stock solutions were frozen and stored in amber multidose containers under nitrogen.

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

The disappearance of folic acid or methotrexate from the gut lumen was followed by removing 0.1-ml samples at 5-min intervals, diluting the

[1] Charles Rivers, Wilmington, Mass.
[2] Bristol Laboratories, Syracuse, N.Y.
[3] Abbott Laboratories, North Chicago, Ill.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Lederle Laboratories, Pearl River, N.Y.
[6] Amersham/Searle Corp., Arlington Heights, Ill.

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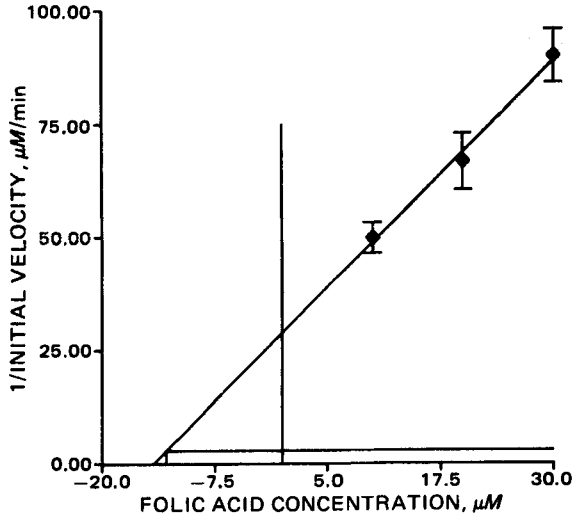

Figure 3—*Reciprocal of initial methotrexate absorption velocity at 8 × 10^{-6} M methotrexate in the presence of 1 × 10^{-5}, 2 × 10^{-5}, and 3 × 10^{-5} M folic acid (mean of four animals ± SD).*

samples in 15 ml of scintillation cocktail [2,5-diphenyloxazole[7] (4 g), 1,4-bis[2-(5-phenyloxazolyl)]benzene[7] (0.1 g), octoxynol[7] (360 g), and toluene[7] (*q.s.* to 1000 ml)], and counting on a scintillation counter[8]. Counting efficiencies were 26% for ^{3}H-methotrexate and 70% for 2-^{14}C-folic acid.

RESULTS AND DISCUSSION

A Lineweaver–Burk plot (10) of the reciprocal of the initial absorption velocity *versus* the reciprocal of the initial folic acid concentration in the lumen of the rat small intestine *in situ* is shown in Fig. 1. With the NONLIN program (11) and a digital computer[9], the data were fitted to (12):

$$V = \frac{(V_{max}C)}{(K_m + C)} \qquad \text{(Eq. 1)}$$

The values of V_{max} and K_m were 4.63 ± 0.3 × 10^{-7} M/min and 1.21 ± 0.21 × 10^{-6} M, respectively. These values were used to generate the solid line shown in Fig. 1.

The V_m values for methotrexate and folic acid (4.78 × 10^{-7} and 4.63 × 10^{-7} M/min) were quite similar; however, the K_m values obtained *in vivo* (1.49 × 10^{-5} and 1.21 × 10^{-6} M) were significantly different. This result is similar to earlier work by Sirotnak and coworkers (2–4) using isolated cell preparations, which also demonstrated differences in K_m with changes in chemical structure but relatively little change in V_m. In the present work, differences in transport rate apparently were due also to differences in the affinity of the drug for the transport carrier.

Figure 2 shows plots of the reciprocal of the initial folic acid absorption velocity at 1 × 10^{-6} and 2.5 × 10^{-6} M folic acid in the presence of 1 × 10^{-5}, 2 × 10^{-5}, and 3 × 10^{-5} M methotrexate. The plot shows typical competitive inhibition. The intersection of the least-squares lines in Fig. 2 gave a value of 1.9 × 10^{-5} M for the K_i of the methotrexate–transport carrier complex (13).

A plot of the reciprocal of the initial methotrexate absorption velocity at 8 × 10^{-6} M methotrexate in the presence of 1 × 10^{-5}, 2 × 10^{-5}, and 3 × 10^{-5} M folic acid is presented in Fig. 3. Since the competitive nature of the methotrexate inhibition of folic acid was already established, the method of Dixon (14) was used to determine the K_i for the folic acid–transport carrier complex. With the data in Fig. 3, this value was found to be 1.28 × 10^{-6} M.

These results show that methotrexate and folic acid mutually inhibit one another's absorption from the rat small intestine. If similar inhibition occurs in humans, the presence of certain foods, multivitamin preparations, and other sources of relatively large amounts of folic acid may significantly decrease the oral absorption of therapeutic methotrexate doses. Furthermore, oral methotrexate administration in frequent small doses may significantly decrease oral folic acid absorption from natural sources, resulting in an increase in the therapeutic activity and/or toxic side effects of methotrexate.

[7] Research Products International Corp., Elk Grove Village, Ill.
[8] Model 3375, Packard Instrument Co., Downers Grove, Ill.
[9] IBM 370/165.

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