Biochemical Pharmacology, Vol. 44, No. 9, pp. 1839–1842, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

DRUG INTERACTIONS IN INTESTINAL TRANSPORT OF FOLIC ACID AND METHOTREXATE

FURTHER EVIDENCE FOR THE HETEROGENEITY OF FOLATE TRANSPORT IN THE HUMAN SMALL INTESTINE

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(*Received* 8 *May* 1992; *accepted* 30 *June* 1992)

Abstract—The effect of sulfasalazine and olsalazine on the transport of [^{3}H]folic acid and of [^{3}H]-methotrexate (MTX) was investigated in organ-cultured endoscopic biopsy specimens of small intestinal mucosa from normal subjects. Biopsy specimens obtained from patients undergoing routine diagnostic upper gastrointestinal endoscopy were organ-cultured at pH 5.5 and the effect of these two drugs on the initial rate of uptake of the two folates was determined. Both drugs inhibited the transport of [^{3}H]-folic acid with similar K_i values (1.38 and 1.32 mM for sulfasalazine and olsalazine, respectively). However, the uptake of [^{3}H]MTX was only partially inhibited by sulfasalazine and was unaffected by olsalazine. Sulfasalazine inhibited 26.2% of the total flux of MTX, in close agreement with the fraction of MTX flux that has been shown previously to be inhibited by folic acid. These data corroborate previous findings of heterogeneity of transport of MTX in the mucosa of the human small intestine.

The intestinal transport of folate is a complex process that begins with hydrolysis of polyglutamyl folates present in food to monoglutamyl folates. This step is carried out by folyl conjugase, an enzyme present in the intestinal mucosa [1]. The released monoglutamyl folates are transported across the luminal membrane, the rate-limiting step in the overall process of digestion and absorption of dietary folates [2]. We have demonstrated recently distinct carriers for folic acid in the organ-cultured mucosa of the human intestine: active transport across the duodenojejunal mucosa is mediated via two proton-dependent carriers while facilitated diffusion through a low-affinity carrier accounts for the uptake of folic acid in the mucosa of the colon and also in the proximal small intestine under neutral or alkaline pH conditions [3]. In studies using methotrexate (MTX†), a synthetic folate analog, we have further demonstrated the existence of two pathways of intestinal transport of this agent: one is shared with folic acid and mediates some 37% of the total flux of MTX, and the other is not shared with folic acid and is responsible for the major part of MTX transported across the mucosa of the human small intestine [4].

Sulfasalazine (salicylazosulfapyridine), a drug widely used in the treatment of inflammatory bowel diseases, has been demonstrated to impair both the intraluminal hydrolysis of polyglutamyl folates [5] as well as the intestinal transport of folic acid in the human intestine [5, 6]. A related drug, olsalazine (disodium azo disalicylate) is also a competitive inhibitor of folic acid transport in the rat small intestine (Zimmerman J, unpublished observations). The purpose of the present study was, therefore, to elucidate the effect of these drugs on the transport of two folates, folic acid and MTX, in the organ-cultured mucosa of the human small intestine.

MATERIALS AND METHODS

The protocol of this study has been reviewed and approved by the Human Research Review Committee of the Hadassah University Hospital. The experimental procedures and analytical methods were described previously in detail [3, 4]. Briefly, biopsy specimens from the duodenojejunal mucosa were procured, after informed consent, from patients undergoing routine upper gastrointestinal endoscopy for diagnostic purposes. None of the patients had diarrhea or any clinical evidence of malabsorption. In all patients, the mucosa was found to be normal. From each subject, four to six biopsy specimens were procured, and these biopsies were used for a single experiment.

The biopsy specimens were mounted, mucosal surface up, on a stainless steel screen and were cultured at 37° in an atmosphere of 5% CO_2, 95% room air. The culture medium was RPMI 1640 (Bio Lab, Israel), supplemented with glutamine and 10% fetal calf serum. The pH was adjusted to 5.5 with phosphate buffer, 0.15 M. After 20 min, the medium was removed and the tissue was incubated in the same medium containing [^{3}H]folic acid or [^{3}H]MTX (~1.0 μCi/nmol, 0.1 μmol/L), inulin[^{14}C]carboxylic acid (~0.05 μCi/mL) as a marker of the extracellular volume [7], and either sulfasalazine or olsalazine at various concentrations. After exposure to the radioactive medium, the tissue was blotted, weighed, and homogenized in 1.0 mL of water. Aliquots of the tissue homogenate were dissolved in scintillation fluid and counted for 5 min in a liquid scintillation

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† Abbreviations: MTX, methotrexate; ICF, intracellular fluid.

spectrometer programmed to count ^{3}H and ^{14}C radioactivities simultaneously. The intracellular fluid (ICF) volume was calculated as the difference between the total water content of the tissue (83.0% of the wet tissue weight) and the volume of the extracellular fluid (=inulin space). Uptake of the radiolabeled folate into the ICF compartment was calculated from the ^{3}H counts in the tissue homogenate, the [^{14}C]inulin space and the tissue weight. The results were expressed in fmol/μL ICF/10 min. In previous studies we have validated that the counts in the tissue were associated with folic acid or MTX, and we have demonstrated a linearity of uptake for at least 20 min of incubation [3, 4].

Statistical analysis. Each of the experiments was implemented on biopsy specimens from at least 5 different subjects. Data reported are means ± SEM. Linear regression analysis was performed using the least-squares method. The significance of the regression was evaluated by the t statistic [8]. Comparison of the mean uptake rates between different experimental conditions was carried out using one-way analysis of variance, followed by the Scheffé's multiple contrasts procedure or the Dunnett's test as appropriate [8]. In comparison of means of two groups, t-tests were performed. All statistical tests were two-tailed with α less than 5%.

Materials. [^{3}H]Folic acid (42 Ci/mmol), [^{3}H]-MTX (20 Ci/mmol) and inulin[^{14}C]carboxylic acid (9.0 mCi/mmol) were purchased from Amersham Radiochemicals (Amersham laboratories, Buckinghamshire, U.K.) and were purified by paper chromatography prior to use as described previously [3, 4]. The purity of inulin[^{14}C]carboxylic acid was 99% by paper chromatography. Olsalazine was a gift from Pharmacia (Uppsala, Sweden). Stock solutions of the drugs used in these studies were prepared in dimethyl sulfoxide. All other chemicals were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and were of the highest grade available.

RESULTS

In Fig. 1, the effect of sulfasalazine (A) and olsalazine (B) on the initial rate of uptake of folic acid is shown. The Dixon plots demonstrate a competitive inhibition of folic acid uptake with an inhibition constant (K_i) of 1.38 mM for sulfasalazine and 1.32 mM for olsalazine. The effect of these drugs on the transport of MTX was different, as is evident from Fig. 2. Although the initial rate of uptake of MTX was inhibited by sulfasalazine, the dose–response was different as shown in Fig. 2A. A maximal inhibition was achieved at a concentration of 0.125 mmol/L. The difference between the initial rates of transport of MTX in the absence of sulfasalazine and the transport rates in the presence of each of the sulfasalazine concentrations used was significant ($P < 0.05$). However, the differences in transport rates in the presence of the various concentrations of sulfasalazine were not significant. Sulfasalazine inhibited only 26.2% of the total flux of MTX. By contrast, olsalazine had no effect on the initial rate of transport of MTX (Fig. 2B).

DISCUSSION

Administration of sulfasalazine to patients with inflammatory bowel disease has been associated with megaloblastic anemia due to folic acid deficiency [6]. This complication has also been reported in rheumatoid arthritis patients [9]. Moreover, it has

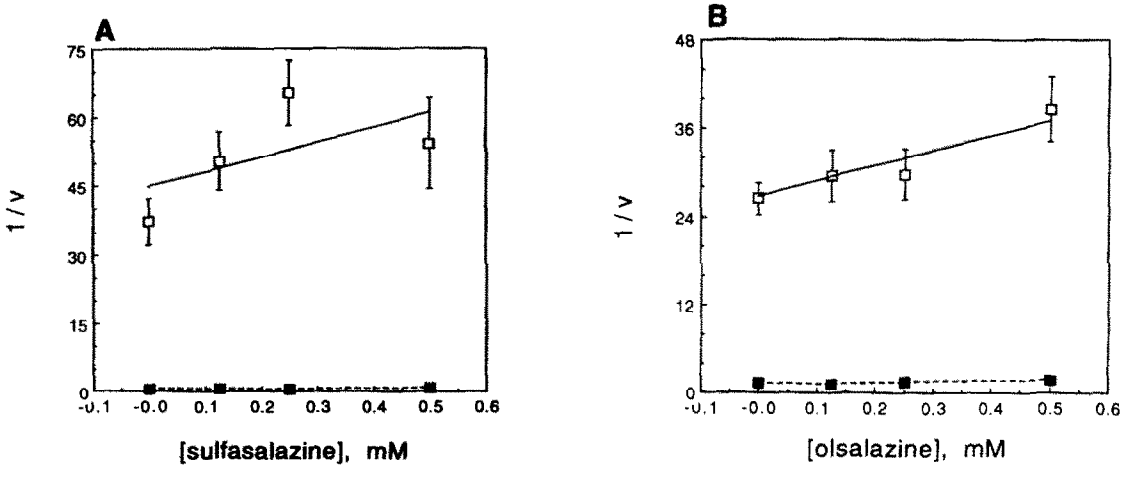

Fig. 1. Effect of sulfasalazine (A) and olsalazine (B) on the transport of folic acid in the organ-cultured mucosa of the human proximal small intestine at pH 5.5-Dixon plots. The data are means of five experiments under each condition. The bars indicate SEM; where bars are absent, SEM merge into the data points. The lines indicate least-squares fits of a linear model to the data at folic acid concentration 0.1 μmol/L (empty squares, continuous lines) and 10 μM (solid squares, broken lines). The slopes of the regression lines at folic acid concentration 0.1 μmol/L are significantly different from zero ($P < 0.05$), whereas those at folic acid concentration 10 μmol/L are not.

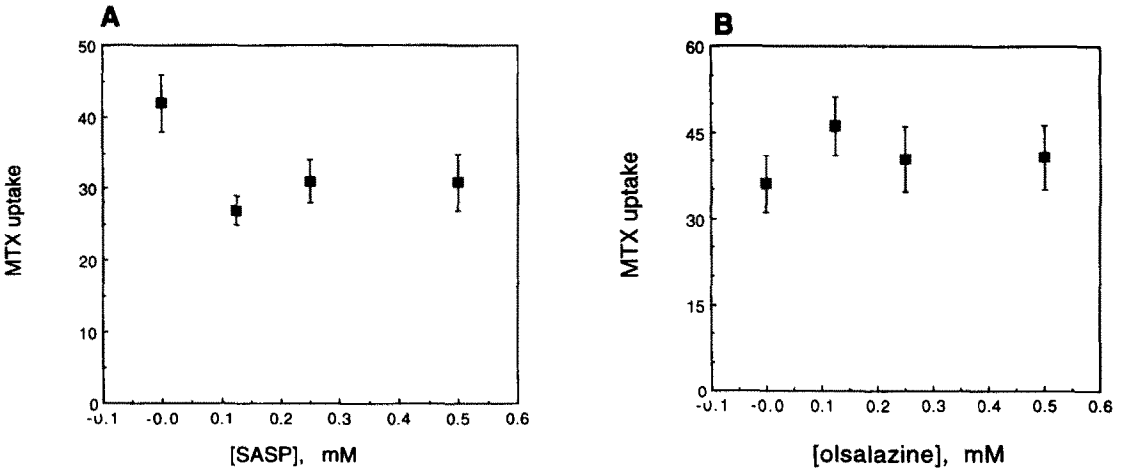

Fig. 2. Effect of sulfasalazine (A) and olsalazine (B) on transport of MTX in the organ-cultured mucosa of the human proximal small intestine at pH 5.5. The initial rate of transport is expressed in fmol/μL intracellular fluid/10 min. The data are means of 16 experiments for sulfasalazine and 12 experiments for olsalazine. MTX concentration was 0.1 μmol/L.

been proposed that sulfasalazine may cause a deficiency of folic acid in the colonic mucosa of patients with ulcerative colitis [10, 11]. This localized deficiency may be a possible causative factor in the dysplastic changes which take place in the mucosa of patients with long-standing disease, eventually culminating in frank colon cancer [11]. Olsalazine, by contrast, has not been associated with folic acid deficiency in the human. However, experience with this drug in clinical practice is still rather limited. After administration of 1 g of sulfasalazine orally, a common therapeutic dose, the concentration of this drug in the proximal small intestine, where the major part of folic acid is actively absorbed, should be 2.5 mmol/L, assuming dilution in 1.0 L. This concentration is almost 2-fold higher than the K_i for transport of folic acid in the organ-cultured mucosa of the human intestine. Since the therapeutic dose of olsalazine is smaller, this drug is much less likely to achieve intraluminal concentrations sufficient to impair significantly the absorption of folic acid.

The effect of sulfasalazine and olsalazine on the transport of folic acid and MTX was investigated at a medium pH of 5.5, which is not only a physiological pH in the human proximal small intestine in the post prandial state [12] but is also the optimal pH for the transport of these two folates [3, 4]. Both sulfasalazine and olsalazine inhibited competitively the transport of folic acid, with a similar inhibition constant. However, the uptake of MTX was only partially inhibited by sulfasalazine and was unaffected by olsalazine. Thus, the uptake of the two folates was affected in different ways by the two drugs. We have shown previously that the transport of MTX in the human proximal small intestine proceeds via two separate routes: about one third of the total flux of MTX is shared with folic acid while the major component of MTX flux is not inhibited by folic acid [4]. In the present study we have found that about 26% of the total flux of MTX was inhibited by sulfasalazine. Thus, it is possible to partition the total flux of MTX into two components: a sulfasalazine-sensitive flux and a sulfasalazine-insensitive flux, the latter constituting the major component of MTX flux in the human small intestine. The close agreement between the proportions of the folic acid-sensitive flux and the sulfasalazine-sensitive flux suggests that these fluxes represent a similar if not identical pathway for the intestinal uptake of MTX, which is shared by folic acid. Thus, the drugs employed in the present study may be useful probes to study the heterogeneity of intestinal folate transport.

Acknowledgements—The present research was supported in part by Federal Funds from the U.S. Dept. of Agriculture, Agricultural Research Service, under contract No. 53-3K06-5-10. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products or organizations imply endorsement by the U.S. government.

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