Inhibitory effect of unconjugated bile acids on the intestinal transport of 5-methyltetrahydrofolate in rat jejunum *in vitro*

H M SAID, D HOLLANDER, AND W B STRUM

From the Department of Medicine, University of California, Irvine, California, and Scripps Clinic and Research Foundation, LaJolla, California, USA

SUMMARY The effect of the unconjugated bile acids, cholic, deoxycholic, chenodeoxycholic, and ursodeoxycholic acids, and of the conjugated bile acid taurocholic acid on the mucosal-to-serosal transport and tissue uptake of the naturally occurring folate derivative, 5-methyltetrahydrofolate (5-CH₃H₄PteGlu) was examined in everted sacs of rat jejunum. Each of the unconjugated bile acids examined inhibited the transport and tissue uptake of 5-CH₃H₄PteGlu in a concentration dependent manner. At low concentrations (0.01-0.1 mM) of cholic and deoxycholic acids, no structural or functional damage to the intestinal mucosa occurred and the transport of 5-CH₃H₄PteGlu was inhibited competitively with K_i values of 0.114 mM and 0.055 mM for cholic and deoxycholic acids, respectively. The greater inhibition of 5-CH₃H₄PteGlu transport by unconjugated bile acids at 1 mM can be attributed to observed structural and functional damage to the intestinal mucosa. The addition of 2 mM lecithin to the mucosal medium failed to prevent the inhibitory effect of 0.1 mM deoxycholic acid on the transport of 0.5 μM 5-CH₃H₄PteGlu. Compared with the effect of unconjugated bile acids, the conjugated bile acid taurocholic acid (0.01-5 mM) showed no effect on the transport and tissue uptake of 5-CH₃H₄PteGlu. The results of this study show that intestinal transport and tissue uptake of 5-CH₃H₄PteGlu are inhibited by unconjugated bile acids in a dose-dependent fashion. The clinical and physiological implications of these observations are discussed.

We have shown previously that the transport of the naturally occurring folate derivative 5-methyltetrahydrofolate (5-CH₃H₄PteGlu) by rat jejunum *in vitro* takes place by two systems: (1) an active, carrier-mediated process (K_t=0.3 μM) which is pH, Na⁺, glucose, and energy dependent, and (2) diffusion, which is pH and energy independent and operates at high (>1 μM) concentrations.¹⁻³ These observations have been confirmed in our laboratory *in vivo* in the unanaesthetised rat (unpublished data). Most of the previous studies describing the intestinal transport of 5-CH₃H₄PteGlu and other folates have been performed *in vitro*. Therefore, the effect of intraluminal factors such as unconjugated and conjugated bile acids on the transport process

have not been previously explored. In this study we describe the effect of unconjugated cholic, deoxycholic, chenodeoxycholic and ursodeoxycholic acids and of a conjugated bile acid taurocholic acid on the intestinal transport and tissue uptake of 5-CH₃H₄PteGlu in everted sacs of rat jejunum. We found that unconjugated bile acids are potent inhibitors of the transport and tissue uptake of 5-CH₃H₄PteGlu while the conjugated bile acid taurocholic acid had no effect.

Methods

MATERIALS

The following materials were obtained commercially: unlabelled 5-CH₃H₄PteGlu, cholic acid-sodium salt, deoxycholic acid-sodium salt, chenodeoxycholic acid, ursodeoxycholic acid, taurocholic acid-sodium salt (Sigma Chemical Co); 5-¹⁴CH₃H₄PteGlu-barium salt (specific activity 58

Address for correspondence: Professor H M Said, Vanderbilt University Medical Center, Department of Pediatric Gastroenterology/Nutrition, D-4113 MCN, Nashville, TN, 37232, USA.

Received for publication 24 February 1984

mCi/mmol) (Amersham/Searle Corp, Des Plaines, IL); scintillation cocktail, Aquamix (Western Product). All chemicals were of analytical quality. The radiochemical purity of $5\text{-}^{14}\text{CH}_3\text{H}_4\text{PteGlu}$ was determined on cellulose precoated thin layer chromatography plates using 0.1 M phosphate buffer, pH 7, containing 5% mercaptoethanol and was found to be 98%.

TRANSPORT STUDY

Fed male Sprague-Dawley rats weighing 180–250 g were used. The animals were stunned by a blow to the head followed by cervical dislocation. The abdomen was opened and the proximal jejunum was removed, washed with cold phosphate buffer and divided into four segments of 6 cm each. Everted sacs were then prepared as described previously.¹ The serosal solution consisted of 0.4 ml of the same buffer used for incubation. The sacs were incubated in 10 ml Erlenmeyer flasks containing 6 ml of the incubation medium which was continuously oxygenated with 95% O_2 and 5% CO_2 . The incubation was carried out at 37°C in a shaking water bath at 80 oscillations per minute. Incubation time of 30 minutes was based on our previous studies which showed that the rate of transport and tissue uptake of $5\text{-CH}_3\text{H}_4\text{PteGlu}$ remained linear throughout this time period.¹⁴ The sacs were then removed and washed and the serosal medium was drained into a scintillation vial containing 6 ml of the scintillation cocktail. After the tissue had dried for 12 hours at 60°C, it was combusted in a biological material oxidizer (Packard model B 306, Packard Instruments Company, IL) and its $^{14}\text{CO}_2$ was collected for radioactivity determination.

The incubation medium contained 110 mM NaH_2PO_4 , 35.2 mM NaCl, 5.5 mM KCl, 1.8 mM MgSO_4 and 20 mM glucose. Sodium ascorbate (3 mg/ml) was added to the incubation medium as an antioxidant. The pH of the incubation medium was adjusted to 6.1 with 1 M NaOH. Labelled and unlabelled $5\text{-CH}_3\text{H}_4\text{PteGlu}$ and the bile acid under investigation were added to the incubation medium at the onset of the experiment. Cholic acid, deoxycholic acid and taurocholic acid formed clear solutions, while chenodeoxycholic acid and ursodeoxycholic acid were only partially soluble.

Results

The effect of unconjugated cholic, deoxycholic, chenodeoxycholic, and ursodeoxycholic acids on the mucosal-to-serosal transport of $5\text{-CH}_3\text{H}_4\text{PteGlu}$ is shown in Table 1. All the unconjugated bile acids used in this study inhibited the mucosal-to-serosal transport of $5\text{-CH}_3\text{H}_4\text{PteGlu}$. The inhibition

Table 1 Effect of the unconjugated cholic, deoxycholic, chenodeoxycholic and ursodeoxycholic acids on the mucosal-to-serosal transport of 0.1 and 0.5 μM $5\text{-CH}_3\text{H}_4\text{PteGlu}$. Jejunal everted sacs were incubated in phosphate buffer pH 6.1 for 30 min at 37°C

Bile acid	Conc (mM)	Mucosal-to-serosal transport (nmol/g initial tissue wet wt/30 min)	
		5- $\text{CH}_3\text{H}_4\text{PteGlu}$ conc (μM)	
		0.1	0.5
Cholic acid	Control	0.064 $\pm$ 0.010(6)	0.30 $\pm$ 0.030(6)
	0.01	0.049 $\pm$ 0.010(6)	0.25 $\pm$ 0.040(6)
	0.05	0.040 $\pm$ 0.004(3)	0.22 $\pm$ 0.004(3)
	0.10	0.032 $\pm$ 0.002(6)	0.15 $\pm$ 0.010(6)
	0.50	0.021 $\pm$ 0.001(3)	0.14 $\pm$ 0.020(3)
	1.00	0.015 $\pm$ 0.001(6)	0.08 $\pm$ 0.020(6)
Deoxycholic acid	Control	0.043 $\pm$ 0.001(6)	0.25 $\pm$ 0.040(6)
	0.01	0.030 $\pm$ 0.002(6)	0.19 $\pm$ 0.060(6)
	0.05	0.020 $\pm$ 0.001(3)	0.14 $\pm$ 0.030(3)
	0.10	0.015 $\pm$ 0.001(3)	0.12 $\pm$ 0.020(6)
	0.50	0.015 $\pm$ 0.001(3)	0.12 $\pm$ 0.010(3)
	1.00	0.012 $\pm$ 0.003(6)	0.07 $\pm$ 0.010(6)
Chenodeoxycholic acid	Control		0.25 $\pm$ 0.030(3)
	0.01		0.25 $\pm$ 0.060(3)
	0.10		0.18 $\pm$ 0.020(3)
	1.00		0.12 $\pm$ 0.010(3)
Ursodeoxycholic acid	Control		0.24 $\pm$ 0.030(3)
	0.01		0.20 $\pm$ 0.010(3)
	0.10		0.09 $\pm$ 0.010(3)
	1.00		0.06 $\pm$ 0.010(3)

Values are mean $\pm$ SEM.

The number of experiments at each specific point is indicated in parentheses.

increased as the concentration of the unconjugated bile acids in the mucosal medium was increased. Using a plot of the reciprocal of the mucosal-to-serosal transport rate of 0.1 and 0.5 μM $5\text{-CH}_3\text{H}_4\text{PteGlu}$ against the inhibitor concentrations of 0.1 mM and lower,⁵ we found a competitive type of inhibition with K_i values of 0.114 mM and 0.055 mM for cholic acid and deoxycholic acid, respectively (Fig. 1a, b). Tissue uptake of $5\text{-CH}_3\text{H}_4\text{PteGlu}$ was inhibited also by the unconjugated bile acids in a concentration dependent manner (Table 2).

The addition of 2 mM lecithin to the mucosal medium did not protect the $5\text{-CH}_3\text{H}_4\text{PteGlu}$ transport system against the inhibitory effect of 0.1 mM deoxycholic acid. Mucosal-to-serosal transport rates of 0.5 μM $5\text{-CH}_3\text{H}_4\text{PteGlu}$ of 0.35 $\pm$ 0.07, 0.17 $\pm$ 0.02 and 0.16 $\pm$ 0.01 nmol/g initial tissue wet wt/30 min were observed for untreated, 0.1 mM deoxycholic acid treated, and 0.1 mM deoxycholic acid plus 2 mM lecithin treated sacs, respectively.

At the concentrations used in these studies (0.01–5 mM), the conjugated bile acid taurocholic acid showed no effect on the transport or tissue uptake of 0.5 μM $5\text{-CH}_3\text{H}_4\text{PteGlu}$ (Table 3).

Figure Competitive inhibition of 5-CH₃H₄PteGlu transport by: (a) cholic acid, and (b) deoxycholic acid. Velocity (*v*) was expressed as the rate of 5-CH₃H₄PteGlu mucosal-to-serosal transport in nmol/g initial tissue wet wt/30 min. Experiments were performed using jejunal everted sacs incubated in phosphate buffer pH 6.1 for 30 min at 37°C.

Table 2 Effect of the unconjugated cholic, deoxycholic, chenodeoxycholic and ursodeoxycholic acids on tissue uptake of 0.1 and 0.5 μM 5-CH₃H₄PteGlu. Jejunal everted sacs were incubated in phosphate buffer pH 6.1 for 30 min at 37°C

Bile acid	Conc (mM)	Tissue uptake (nmol/g initial tissue wet wt/30 min)	
		5-CH ₃ H ₄ PteGlu conc (μM)	
		0.1	0.5
Cholic acid	Control	0.26±0.10(6)	1.45±0.40(6)
	0.01	0.20±0.02(6)	1.13±0.40(6)
	0.05	0.12±0.02(3)	0.95±0.03(3)
	0.10	0.10±0.01(6)	0.85±0.20(6)
	0.50	0.08±0.02(3)	0.62±0.07(3)
	1.00	0.05±0.03(6)	0.40±0.06(6)
Deoxycholic acid	Control	0.29±0.01(6)	1.21±0.10(6)
	0.01	0.23±0.02(6)	0.96±0.20(6)
	0.05	0.16±0.04(3)	0.73±0.10(3)
	0.10	0.13±0.03(6)	0.64±0.07(6)
	0.50	0.14±0.02(3)	0.42±0.13(3)
	1.00	0.10±0.03(6)	0.40±0.01(6)
Chenodeoxycholic acid	Control		1.24±0.12(3)
	0.01		1.12±0.15(3)
	0.10		1.13±0.13(3)
	1.00		0.71±0.07(3)
Ursodeoxycholic acid	Control		1.24±0.12(3)
	0.01		1.02±0.12(3)
	0.10		0.54±0.07(3)
	1.00		0.31±0.05(3)

Values are mean±SEM.

The number of experiments at each specific point is indicated in parentheses.

Table 3 Effect of the conjugated bile acid taurocholate on the mucosal-to-serosal transport and tissue uptake of 0.5 μM 5-CH₃H₄PteGlu. Jejunal everted sacs were incubated in phosphate buffer pH 6.1 for 30 min at 37°C

Taurocholate conc (mM)	Transport (nmol/g initial tissue wet wt/30 min)	Tissue uptake (nmol/g initial tissue wet wt/30 min)
Control	0.26±0.03(6)	1.31±0.30(6)
0.01	0.26±0.04(4)	1.27±0.20(4)
0.10	0.25±0.07(4)	1.35±0.40(4)
1.00	0.25±0.04(4)	1.25±0.30(4)
5.00	0.24±0.04(4)	1.30±0.10(4)

Values are mean±SEM.

The number of experiments at each specific point is indicated in parentheses.

Discussion

Our data show that unconjugated cholic, deoxycholic, chenodeoxycholic, and ursodeoxycholic acids are potent inhibitors of the intestinal mucosal-to-serosal transport and tissue uptake of 5-CH₃H₄PteGlu *in vitro*. Low concentrations of cholic and deoxycholic acids (0.01–0.1 mM) inhibit competitively the transport of 5-CH₃H₄PteGlu. Histological studies, transmural potential difference measurements, and the rate of release of the cytoplasmic marker enzyme LDH have shown that at these range of low concentrations neither cholic nor deoxycholic acids cause structural or functional damages to the intestinal mucosa.^{2,4,6} Lecithin, which prevents the toxic and damaging effects of bile acids on biological membranes, including small intestinal epithelium,^{7,8} failed to protect the transport system of 5-CH₃H₄PteGlu against the inhibitory effect of a low concentration (0.1 mM) of deoxycholic acid. This further supports the interpretation that transport inhibition by unconjugated bile acids is not because of damage of the intestinal tissue. On the other hand, higher concentrations (≥1 mM) of the unconjugated bile acids can cause demonstrable structural and functional damage to the intestinal mucosa,^{4,6} thus explaining the increased inhibition of 5-CH₃H₄PteGlu transport at high bile acid concentrations. The addition of the conjugated bile acid, taurocholic acid, at a concentration of 0.01–5 mM to the mucosal medium did not inhibit the transport and tissue uptake of 5-CH₃H₄PteGlu. The contrasting effects of the conjugated and unconjugated bile acids suggest that the carboxyl group of the bile acid must be free in order to allow the bile acid to inhibit the transport of folates and that the free carboxyl group of the bile acid interacts with the transport system of

5-CH₃H₄PteGlu. This hypothesis is in agreement with studies indicating that the active transport process is specific for the intact folate molecule and requires free α - and γ -carboxyl groups on the folate compound.⁹

The observations that unconjugated, but not conjugated, bile acids inhibit the intestinal absorption of 5-CH₃H₄PteGlu are of physiological and clinical importance. Under normal physiological conditions, only conjugated bile acids are secreted by the liver into the small intestinal lumen¹⁰ and folate absorption is unimpaired. In diseases associated with bacterial overgrowth of the proximal small intestine,¹¹⁻¹⁵ deconjugation of bile acids can occur but only rarely does folate malabsorption occur.¹¹⁻¹⁵⁻¹⁷ Inhibition of the intestinal absorption of folates by unconjugated bile acids, however, could provide a partial explanation for the reported folate malabsorption and deficiency seen at times in patients with tropical sprue, partial gastrectomy, scleroderma¹⁸⁻²⁰ and other conditions of intestinal stasis. This mechanism may be in addition to inhibition of folic acid conjugase by unconjugated dihydroxy bile acids observed in guinea pig intestinal mucosa *in vitro*.²¹ Moreover, unconjugated bile acids, namely chenodeoxycholic and ursodeoxycholic acids, in use clinically for the dissolution of gall stones,²² theoretically could interfere with the absorption of folates and folate analogues. A careful assessment of the folate status of patients receiving unconjugated bile acids and the ability of unconjugated bile acids to block intestinal absorption of folate analogues should be carried out in order to determine the clinical importance of our observations.

This study was supported by the US Public Health Service grant AG 2767, the National Cancer Institute grant CA-17809, and the Goldsmith Family Foundation.

Abstract of this work was presented in the annual meeting of the American Gastroenterology Association May 1983, Washington, DC, and appeared in *Gastroenterology* 1983; **84**: 1293.

References

- 1 Said HM, Strum WB. A pH-dependent, carrier-mediated system for transport of 5-methyltetrahydrofolate in rat jejunum. *J Pharmacol Exp Ther* 1983; **226**: 95-9.
- 2 Said HM, Strum WB, Hilburn M, Blair JA. The intestinal surface acid microclimate and folate absorption. *Gastroenterology* 1982; **82**: 1167.
- 3 Strum WB, Said HM. Intestinal folate transport: a pH-dependent, carrier-mediated process. In: Blair JA, ed. *Chemistry and biology of pteridines*. Berlin: Walter de Gruyter and Company, 1983.
- 4 Said HM. In: *The surface properties of the mammalian small intestine, their role in the absorption of nutrients and their variation in health and disease*. Department of Chemistry, University of Aston in Birmingham, Birmingham, UK: PhD Thesis, 1981.
- 5 Dixon M. The determination of enzyme inhibitor constants. *Biochem J* 1953; **55**: 170-1.
- 6 Said HM, Hollander D, Strum WB. The enterohepatic circulation of methotrexate. Effect of unconjugated bile salts. *Proc Am Ass Cancer Res* 1983; **24**: 275.
- 7 Wingate DL, Phillips SF, Hofmann A. Effect of glycine-conjugated bile acids with and without lecithin on water and glucose absorption in perfused human jejunum. *J Clin Invest* 1973; **52**: 1230-6.
- 8 Ammon HV. Effect of taurine conjugated bile salts with and without lecithin on water and electrolyte transport in the canine gallbladder *in vivo*. *Gastroenterology* 1979; **76**: 778-83.
- 9 Strum WB. Characteristics of the transport of pteroylglutamate and amethopterin in rat jejunum. *J Pharmacol Exp Ther* 1981; **216**: 329-33.
- 10 Hofmann AF. The enterohepatic circulation of bile acids in man. *Clin Gastroenterol* 1977; **6**: 1-24.
- 11 Tabaqchali S, Booth CC. Jejunal bacteriology and bile salt metabolism in patients with intestinal malabsorption. *Lancet* 1966; **2**: 12-15.
- 12 Kahn IJ, Jeffries GH, Sleisenger MH. Malabsorption in intestinal scleroderma. *N Engl J Med* 1966; **274**: 1339-44.
- 13 Gorbach SL, Mitra R, Jacobs B *et al*. Bacterial contamination of the upper small bowel in tropical sprue. *Lancet* 1969; **1**: 74-7.
- 14 Isaacs PET, Kim YS. Blind loop syndrome and small bowel bacterial contamination. *Clin Gastroenterol* 1983; **12**: 395-414.
- 15 Simon GL, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology* 1984; **86**: 174-93.
- 16 Hill MJ. Some leads to the etiology of cancer of the large bowel. *Surg Annu* 1978; **10**: 135-49.
- 17 Lifshitz F, Waphir RA, Wehman HJ, Diaz-Bensussen S, Pergolizzi R. The effect of small intestinal colonization by fecal and colonic bacteria on intestinal function in rats. *J Nutr* 1978; **108**: 1913-23.
- 18 Barrett CR, Holt PR. Postgastrectomy blind loop syndrome. Megaloblastic anemia secondary to malabsorption of folic acid. *Am J Med* 1966; **41**: 629-37.
- 19 Rosenberg IH. Absorption and malabsorption of folates. *Clin Haematol* 1976; **5**: 589-618.
- 20 Lindenbaum J. Aspects of vitamin B₁₂ and folate metabolism in malabsorption syndromes. *Am J Med* 1979; **67**: 1037-48.
- 21 Bernstein LH, Gutstein S, Weiner S. Folic acid conjugase: inhibition by unconjugated dihydroxy bile acids. *Proc Soc Exp Biol Med* 1969; **132**: 1167-9.
- 22 Schoenfield LJ, Lachin JM. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones: the national cooperative gallstone study. *Ann Intern Med* 1981; **95**: 257-82.