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The enterohepatic circulation of methotrexate in vivo: Inhibition by bile salt*

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Summary. We examined the enterohepatic circulation of methotrexate (MTX) in the rat in vivo and determined the effect of the unconjugated bile salt, cholate, on the process. MTX (70 mg/kg body weight) was administered i. v. and the bile salt (1 m*M*) was delivered through intestinal perfusion. In the control group 38.43%±4% of the administered dose of MTX appeared in bile 2 h after administration of the drug. In the bile salt-treated group 21.4%±3.7% of the administered does of MTX appeared in bile, which was significantly lower ($P<0.01$) than the proportion in the control group. The liver content of MTX was depressed by 23% in the bile salt-treated group compared with the control group. This study demonstrates, in vivo, the important role that the enterohepatic circulation plays in exposing the small intestine to toxic levels of MTX and shows that the unconjugated bile salt, cholate, inhibits the process.

Introduction

The folate antagonist methotrexate (MTX) is used extensively in the treatment of a wide variety of neoplastic diseases [1, 5, 10]. The limiting factor in the therapeutic use of MTX is its toxicity for proliferating cells, especially the rapidly dividing cells of the intestinal crypts [1]. The toxicity is thought to be potentiated by prolonged retention of MTX in the body because of the enterohepatic circulation [1, 5, 11].

Previous studies from our laboratory using in vitro intestinal and liver preparations have shown that unconjugated bile salts at nontoxic concentrations interrupt MTX enterohepatic circulation by inhibiting its intestinal transport, liver uptake, and biliary excretion [8]. Our recent in vivo study [6] on the effect of bile salts on MTX intestinal absorption confirms the previously reported in vitro observation. In this study we examined the enterohepatic circulation of MTX in vivo in the rat and determined the effect of the unconjugated bile salt, cholate, on MTX biliary excretion and liver uptake.

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Materials and methods

Materials. The following were obtained commercially: unlabeled MTX and cholic acid-sodium salt from Sigma Chemical Company. St Louis Mo; [3, 4- ^{3}H] MTX (47 Ci/mmol) from New England Nuclear', Mass; ACS scintillation cocktail from Amersham, Arlington Heights, Ill; cellulose-precoated thin-layer chromatograms from Eastman Kodak Company, Rochester, NY. All other chemicals and reagents were of analytic quality. The radiolabeled MTX was purified before use on precoated cellulose thin-layer chromatography plates, using a 160-m*M* phosphate buffer, pH 7.0, as the solvent system. The purified ^{3}H-MTX used in this study was 96% radiochemically pure.

Methods. Unfasted male Sprague-Dawley rats (Sasco, Omaha, Neb) weighing 300–380 g were used in this study. Rats were anesthetized with Nembutal (45 mg/kg body weight, i. p.) and kept on heated surgical pads. The abdomen was opened and the common bile duct was cannulated with polyethylene tubing. The small intestine was then cannulated, as described previously [7], from the proximal duodenum to the distal ileum. The intestinal segment was flushed with Krebs-Ringer phosphate buffer (20 m*M* NaH_2PO_4, 125 m*M* NaCl, 4.93 m*M* KCI, 1.23 m*M* $MgSO_4$, 0.85 m*M* $CaCl_2$, 10 m*M* glucose; pH 7.0) then perfused for 2.5 h with the same buffer in the absence (control group) and presence (treated group) of 1 m*M* cholate. At 30 min after the start of the intestinal perfusion, 0.9 ml saline solution containing MTX 70 mg/kg body weight and ^{3}H-MTX 20 μCi was injected through the femoral vein. Bile was collected over a period of 2 h following injection. Then, the rats were killed with an overdose of ether and the liver was removed and weighed. The liver was cut into small pieces and dropped into a boiling 50 m*M* phosphate buffer pH 7.0 (wt/vol ⅓). After boiling for 10 min, the mixture was allowed to cool. It was then homogenized for 5 min in a Waring blender-type homogenizer at maximum speed and centrifuged at 30 000 rpm for 30 min (L3-50 Ultracentrifuge, Beckman Instruments, Inc., Fullerton, Calif). A measured amount (0.5 ml) of the supernatant was taken and added to a scintillation vial containing 0.5 ml distilled water and 10 ml scintillation cocktail. Samples were then counted for radioactivity in a scintillation counter (model LS 3801, Beckman Instruments, Irvine, Calif). The amount of radioactivity that appeared in the bile was determined by adding 50 μl bile to a scintillation vial con-

Table 1. Biliary excretion[a] and liver uptake[a] of MTX 2 h following administration of 70 mg/kg body weight of MTX in control and bile salt-treated rats

	Control group	Bile salt-treated group
Biliary excretion	38.43±4.1 (4)	21.40±3.4 (6)
Liver content	1.93±0.3 (4)	1.49±0.2 (6)
Bile volume[b]	1.16±0.12 (4)	1.69±0.17 (6)

[a] Expressed as percentages (means ± SE) of the administered doses with number of experiments shown in parentheses in each case

[b] ml/2 h

taining 0.95 ml distilled water and 10 ml scintillation cocktail and counted for radioactivity.

Each datum presented in this paper is the mean ±SE of at least four separate observations. Data were analyzed using Student's *t*-test.

Results

Table 1 shows the results of biliary excretion and liver content of MTX 2 h after the administration of MTX 70 mg/kg body weight i. v. to control rats (the small intestine was perfused for 2.5 h with Krebs-Ringer phosphate buffer) and bile salt-treated rats (the small intestine was perfused for 2.5 h with Krebs-Ringer phosphate buffer containing 1 m*M* cholate). In the control group, 38.43%±4% of the administered dose of MTX appeared in bile 2 h after administration of the drug. In the bile salt-treated group, 21.4%±3.7% of the administered dose of MTX appeared in bile, which was significantly lower ($P<0.01$) than the proportion in the control group. The bile volume was significantly higher ($P<0.01$) in the bile salt-treated group than in the control group. The correlation between bile volume and MTX biliary excretion was examined using the nonparameteric Spearman – Rank method and found to be nonsignificant ($P=0.078$). The liver uptake of MTX was also decreased (though not significantly) by 23% in the bile salt-treated group compared with the control group.

Discussion

The present investigation examined the enterohepatic circulation of MTX in vivo following i. v. administration of a single dose of the drug and determined the effect of the unconjugated bile salt, cholate, on the process. Excretion of MTX in bile was extensive in the control group, with 38.43% of the administered dose of the drug appearing in the bile 2 h after administration. This observation confirms the previously reported in vitro studies on the extent of the enterohepatic circulation of MTX [11] and clearly demonstrates the significant role biliary excretion of MTX plays in exposing the intestine to toxic concentrations of the drug. The unconjugated bile salt, cholate, when administered through intestinal perfusion, inhibited both bile excretion and liver uptake of MTX. These observations confirm our previous finding with the isolated, perfused rat liver [8]. Based on in vitro observations on the ability of bile salt to interrupt the enterohepatic circulation of MTX, we have previously proposed that bile salts should be tried therapeutically to decrease MTX intestinal toxicity. Our present in vivo observations clearly support that proposal. The use of bile salt should not interfere with the interaction of MTX with the target tumor site, because: (a) the extremely efficient removal of bile salts from the portal circulation by hepatocytes would remove the majority of bile salts from the general circulation on the first pass through the liver [4], and (b) high concentrations of bile salt are required to inhibit the transport of MTX into tumor cells [3]. There are precedents for the therapeutic use of bile salts, and unconjugated bile salts are currently in clinical use for the dissolution of gallstone [2, 9].

In summary, the present study demonstrates the important role that the enterohepatic circulation of MTX plays in exposing the small intestine to the drug and shows that the unconjugated bile salt, cholate, inhibits the process.

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