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Effect of cyclosporine on colchicine partitioning in the rat liver

Kermit V. Speeg, Alma L. Maldonado

Division of Gastroenterology, Department of Medicine, The University of Texas Health Science Center and A. L. Murphy Memorial VA Medical Center, San Antonio, TX 78284, USA

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Abstract. Colchicine is secreted into bile as a major pathway of elimination. Cyclosporine (CsA) inhibits colchicine biliary secretion. In the present study, the effects of cyclosporine and its vehicle (cremophor) on the partitioning of colchicine across the liver were studied. CsA decreased the colchicine bile/plasma ratio from 484 ± 39 to 53 ± 3 ($P < 0.001$). This effect was due to both a decrease in bile/liver partitioning (control, 35.1 ± 1.2, vs CsA treatment, 9.2 ± 0.5; $p < 0.001$) as well as a decrease in liver/plasma partitioning (conrol, 13.7 ± 0.8, vs CsA treatment, 5.7 ± 0.4; $P < 0.001$). Cremophor also decreased the colchicine bile/plasma ratio (317 ± 19, $P < 0.02$ vs control), but this effect was due to a decrease in the liver/plasma ratio (9.99 ± 0.7, $P < 0.02$ vs control) rather than the bile/liver ratio (31.9 ± 2.1, $P > 0.2$ vs control). Inhibition at the canalicular membrane is consistent with the location of gp-170, the presumed transporter of colchicine.

Introduction

Colchicine is a substrate for the multidrug-resistance transporter P-glycoprotein, or gp-170, in cultured cells [6]. P-glycoprotein has been found immunohistochemically in the canaliculus of the hepatocyte and the brush border of the proximal renal tubule, among other places [15, 17, 18]. Furthermore, inside-out, liver canalicular vesicles have an adenosine 5′-triphosphate (ATP)-dependent transporter that recognizes gp-170 substrates [5]. Colchicine is secreted into bile [13] and urine [14] in vivo in the normal rat. Cyclosporine reverses multidrug resistance in cultured cells [12, 19], and cyclosporine inhibits colchicine secretion into bile [13] and urine [14]. The mechanism is unknown but is presumed to be inhibition of P-glycoprotein. In this study we assessed the effect of cyclosporine on the partitioning of colchicine across the basolateral and canalicular membranes of the liver so as to determine the site of transport inhibition.

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Correspondence to: K. V. Speeg, Department of Medicine, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284, USA

Materials and methods

Chemicals and reagents. Colchicine, β-lumicolchicine, and Cremophor EL (polyoxyethylated castor oil) were obtained from Sigma Chemical Co. (St. Louis, Mo.). Cyclosporine was purchased as Sandimmune IV (Sandoz, Inc., East Hanover, N.J.). Each milliliter contains 50 mg cyclosporine and 650 mg Cremophor, and this solution was diluted 1:10 (v/v) in saline before injection.

Experimental protocol. Animal studies were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind.) weighing 300–400 g were allowed food and water ad libitum. While the rat was under general anesthesia (pentobarbital sodium, 50 mg/kg given i.p.), cannulas (PE-10 tubing) were placed in the femoral artery, femoral vein, common bile duct, and both ureters. The animals were maintained at normal body temperature with a heating pad throughout the experiment. An infusion was started with a solution of mannitol (5%) and saline (0.9%) at a rate of 0.123 ml/min, which achieved constant urine and bile flow within 60 min. At 10 min after the beginning of the infusion, some rats received an i.v. bolus of cyclosporine (CsA, 10 mg/kg, $n = 3$) or cremophor (CsA vehicle; 130 mg/kg is the equivalent dose given with CsA; $n = 3$); control rats ($n = 4$) were not injected at 10 min. After 60 min of infusion a 10-mg/kg colchicine bolus was given i.v. Subsequently, a 10-min bile and urine collection was made, with arterial blood being sampled at the beginning and end of the collection. The rat was then killed and the liver was removed, flushed with saline, and homogenized with 5 vol. 0.9% saline.

Analytical methods. Colchicine levels wer quantitated by high-performance liquid chromatography (HPLC) as previously described [13, 14]. The mobile phase was acetonitrile: water (28:72, v/v) run at a flow rate of 1.0 ml/min through a μBondapak C18 column (Waters Chromatography Division, Millipore Corp., Milford, Mass.), with detection being carried out at 245 nm. Separation was performed at ambient temperature. β-Lumicolchicine was used as the internal standard. Generally, 700 ng

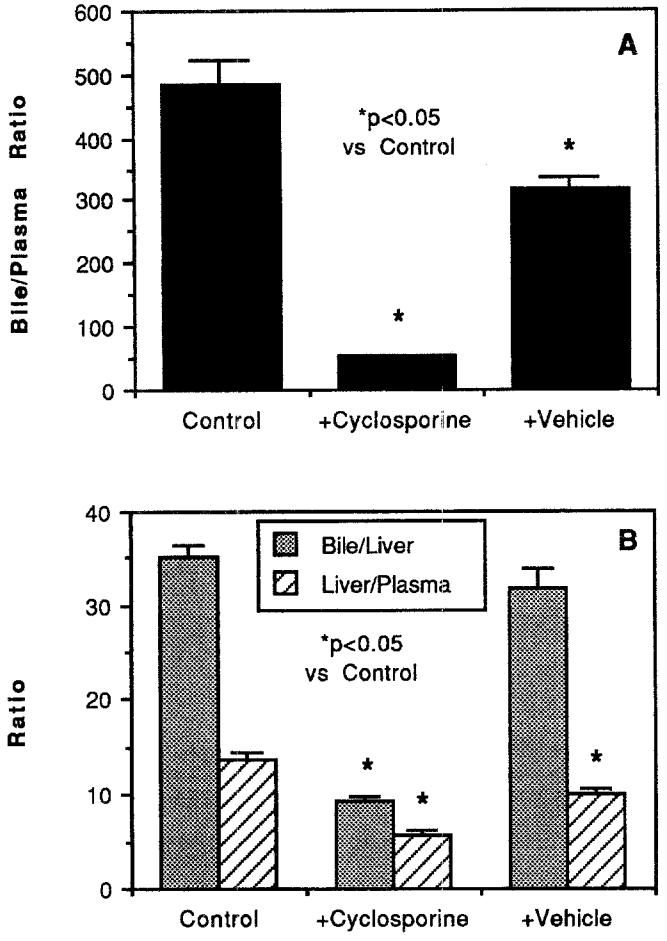

Fig. 1 A, B. Effect of cyclosporine (CsA) on colchicine partitioning in liver. **A** Colchicine bile/plasma ratio in control ($n = 4$), CsA-treated (10 mg/kg, $n = 3$), and vehicle-treated ($n = 3$) rats. Data represent mean values ± SEM. **B** Colchicine bile/liver and liver/plasma ratios in control ($n = 4$), CsA-treated (10 mg/kg, $n = 3$), and vehicle-treated ($n = 3$) rats. Data represent mean values ± SEM

internal standard, 0.5 ml 8 *M* ammonium hydroxide, and 7.0 ml HPLC-grade dichloromethane was added to 50 µl diluted bile (1:1000), 50 µl liver homogenate, or plasma. The mixture was mechanically shaken for 10 min and then centrifuged for 5 min. The aqueous (upper) phase was discarded, the organic (lower) phase was evaporated in a Savant Speed Vac Concentrator, and the residue was redissolved in 200 µl mobile phase.

Statistics and calculations. Data are expressed as mean values ± SEM. Statistically significant differences were tested using analysis of variance (ANOVA), and a P value of <0.05 was accepted as significant. The liver/plasma ratio was calculated as the amount of colchicine in liver at 10 min after injection (micrograms per gram of wet weight) divided by the plasma level of colchicine at 10 min after injection (micrograms per milliliter). The bile/plasma ratio was calculated as the amount of colchicine in bile at 0–10 min after injection (micrograms per milliliter) divided by the plasma level of colchicine at 10 min after injection (micrograms per milliliter). The bile/liver ratio was calculated as bile/plasma divided by liver/plasma.

Results

Bile flow was decreased in the CsA-treated animals but not in those receiving vehicle only (control, 233±13 µl/10 min; CsA, 186±7 µl/10 min (P <0.05); vehicle, 222±11 µl/10 min). Colchicine in bile during the first 10 min after bolus injection was decreased from 515±73 µg in control animals to 60±5 µg (P <0.05) in CsA-treated rats and 267±18 µg (P <0.05) in vehicle-treated rats. Control rats averaged 4.06±0.19 µg colchicine/ml plasma at 10 min after injection, whereas those treated with CsA averaged 6.09±0.13 µg/ml (P <0.05) and those treated with vehicle averaged 3.81±0.12 µg/ml (P <0.05). Liver weights were approximately the same in the three groups (control, 12.37±0.35 g; CsA treatment, 10.79±0.35 g; vehicle, 11.64±0.38 g). Liver colchicine was 51.54±1.74 µg/g liver in controls, 34.95±2.48 µg/g liver in CsA-treated rats (P <0.05), and 38.14±3.46 µg/g liver in vehicle-treated rats ($P \leq 0.05$). The colchicine bile/plasma ratio was 484±39 in controls, 53±3 in CsA-treated rats (P <0.001), and 317±19 in vehicle-treated rats (P <0.02; Fig. 1). The colchicine bile/liver ratio, which might reflect secretion or inhibition of secretion of a canalicular transporter, was 35.1±1.2 in control rats and 9.2±0.5 in CsA-treated rats (P <0.001). In rats treated with vehicle only, the colchicine bile/liver ratio was 31.9±2.1 (not significant). The colchicine liver/plasma ratio, which might reflect concentrative uptake at the basolateral membrane, was 13.7±0.8 in control rats and 5.7±0.4 in CsA-treated rats (P <0.001). This effect may have been partially due to the CsA vehicle, since the colchicine liver/plasma ratio in vehicle-treated rats was 9.99±0.7 (P <0.02).

Discussion

Generally, a drug bile/plasma ratio greater than 10 is considered to be strong evidence of net secretion into bile [10]. In this study, the colchicine bile/plasma ratio was much greater than 10. This ratio consists of two components, the liver/plasma ratio and the bile/liver ratio, and these probe partitioning across the basolateral and canalicular membranes of the liver, respectively.

The bile/liver ratio for colchicine is much larger than the liver/plasma ratio and is consistent with the transporter being located on the canalicular membrane [15, 17, 18]. CsA in vehicle decreased the colchicine bile/plasma ratio by 89%, and this effect was due to both a 74% decrease in bile/liver partitioning and a 58% decrease in liver/plasma partitioning. Vehicle alone resulted in a 35% decrease in the bile/plasma ratio and was due primarily to a 27% decrease in liver/plasma partitioning along with an insignificant 9% decrease in bile/liver partitioning. Thus, the primary site of action of CsA on colchicine partitioning would appear to be the canalicular membrane. The CsA vehicle cremophor appears to affect colchicine partitioning primarily across the basolateral membrane. Vehicles that increase membrane fluidity have been reported to decrease gp-170 substrate movement across canalicular vesicles in vitro [11], and it is possible that a similar mechanism is responsible for the effect of cremophor on the basolateral membrane.

CsA binds to P-glycoprotein [2] and may be a substrate for this transporter in cells expressing high levels of P-gly-

coprotein [3], although we have not been able to demonstrate overall CsA secretion into bile in vivo [13]. CsA also inhibits bile acid secretion [9, 16] and biliary secretion of sulfobromophthalein [1]. Non-P-glycoprotein ATP-dependent transporters have been described in the canalicular membrane for organic anions [4] and bile acids [7], but it is unknown whether these transporters are inhibitable by CsA, thereby accounting for inhibition of secretion. The CsA vehicle cremophor has also been reported to inhibit bile acid secretion and bilirubin secretion [8]. Thus, on the basis of these partitioning studies across the liver, we conclude that the CsA vehicle primarily affects colchicine uptake into liver, whereas CsA primarily affects colchicine secretion into bile.

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