Cancer Chemother Pharmacol (1994) 34: 133–136

Cancer Chemotherapy and Pharmacology

Effect of the nonimmunosuppressive cyclosporin analog SDZ PSC-833 on colchicine and doxorubicin biliary secretion by the rat in vivo

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

Received: 11 August 1993/Accepted: 6 December 1993

Abstract. Colchicine and doxorubicin are secreted into bile as a major pathway of their elimination. Colchicine and doxorubicin are also substrates for P-glycoprotein, and P-glycoprotein has been demonstrated to be present at the liver canalicular membrane. Cyclosporin (CsA) inhibits colchicine biliary secretion in vivo. In the present study, the effects of SDZ PSC-833, a nonimmunosuppressive cyclosporin D analog, on the biliary secretion of colchicine and doxorubicin were investigated. SDZ PSC-833 given at a bolus dose of 2 mg/kg promptly decreased colchicine biliary clearance from 9.05 ± 0.2 to 2.41 ± 0.43 ml min^{-1} kg^{-1} ($P < 0.001$) and the colchicine bile/plasma ratio from 146 ± 8 to 35 ± 5 ($P < 0.001$). SDZ PSC-833 also inhibited doxorubicin biliary clearance (basal: 10.5 ± 3 vs post-SDZ PSC-833: 2.48 ± 0.94 ml min^{-1} kg^{-1}; $P = 0.06$) and the doxorubicin bile/plasma ratio (basal: 228 ± 64 vs post-SDZ PSC-833: 48 ± 22; $P < 0.01$). Colchicine renal secretion was completely inhibited by SDZ PSC-833. Thus, SDZ PSC-833 inhibits the constitutive transport of the multidrug-resistance substrates colchicine and doxorubicin and is more potent than cyclosporin in this regard. The possibility of increased toxicity to normal tissues because of impaired elimination of cytotoxic agents will need to be considered if SDZ PSC-833 is used to chemosensitize cancer cells.

Introduction

Colchicine and doxorubicin are substrates for the multidrug-resistance transporter, P-glycoprotein, or gp-170, in cultured cells [7]. P-glycoprotein has also been found immunohistochemically in the canaliculus of the hepatocyte and the brush border of the proximal renal tubule among other places [13–15]. Furthermore, inside-out, liver canalicular vesicles have an adenosine triphosphate (ATP)-dependent transporter that recognizes gp-170 substrates [5]. Colchicine is secreted into bile [11] and urine [12] in vivo in the normal rat, and doxorubicin is known to be secreted into bile in humans [8]. Cyclosporin reverses multidrug resistance in cultured cells [10, 16] and inhibits colchicine secretion into bile [11] and urine [12]. Recently, a nonimmunosuppressive cyclosporin D analog, SDZ PSC-833, has been reported to reverse multidrug resistance in vitro [1, 3, 6] and in vivo [2, 6]. In the study reported herein, the effect of SDZ PSC-833 on in vivo colchicine and doxorubicin biliary secretion, presumably by constitutive canalicular P-glycoprotein, was investigated.

This work was supported in part by the Research Service, Department of Veterans Affairs

Correspondence to: Dr. 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, doxorubicin, daunomycin, inulin, and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, Mo.). SDZ PSC-833 was a gift from Sandoz Pharmaceutical Co. (East Hanover, N.J.). SDZ PSC-833 was dissolved in DMSO at a concentration of 5 mg/ml.

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 of a solution of mannitol (5%), inulin (0.2%), saline (0.9%), and test drug was started at a rate of 0.123 ml/min, which achieved constant urine and bile flow by 60 min. Colchicine ($n = 3$ rats) was given by continuous infusion at 231 μg min^{-1} kg^{-1}. Doxorubicin ($n = 3$ rats) was given by continuous infusion at 20 μg/min. A 60-min infusion achieved steady-state levels of colchicine and doxorubicin as well as inulin. Subsequently, four serial 10-min bile and urine collections were made, with arterial blood being sampled at the beginning and end of each collection. The rat was then given an i.v. bolus of SDZ PSC-

833 (2 mg/kg in DMSO) and four further bile and urine samples were collected, with arterial blood being sampled at the beginning and end of each collection. Two rats infused with colchicine received DMSO only.

Analytical methods. Colchicine levels were quantitated by high-performance liquid chromatography (HPLC) as previously described [11, 12]. The mobile phase was acetonitrile: water (28: 72, v: v) delivered at a flow rate of 1.0 ml/min through a μBondapak C18 column (Waters chromatography Division, Millipore Corp., Milford, Mass.) and the detection wavelength was 245 nm. Separation was performed at ambient temperature. β-Lumicolchicine was used as the internal standard. Generally, 700 ng 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), 25 μl diluted urine (1: 100), or 50 μl 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. Doxorubicin was quantitated by HPLC. Plasma (50 μl) was mixed with 50 μl acetonitrile and then 10 μl internal standard (daunomycin) in mobile phase and 140 μl mobile phase was added. The mixture was vortexed and centrifuged and the supernatant was injected directly. For urine and bile samples, 10–20 μl was diluted to 1 ml with mobile phase and 20 μl internal standard was added. The mixture was vortexed and injected directly. The mobile phase was 30% acetonitrile-70% ammonium formate (0.1%, pH 4). The flow rate was 1.5 ml/min through a Waters Novapak phenyl column, and fluorescence detection was carried out using an excitation wavelength of 475 nm and an emission wavelength of 580 nm). Inulin was quantitated colorimetrically [4].

Statistics and calculations. Data are expressed as mean values ±SEM. The averages given in the text represent the average of all measurements recorded during the basal period (60–70, 70–80, 80–90, and 90–100 min) for all of the animals (n) in a particular group and all measurements recorded during the period following drug treatment (100–110, 110–120, 120–130, and 130–140 min) for the same animals. Data for each period are shown in the figures. Statistically significant differences were tested analysis of variance using (ANOVA) and a P value of <0.05 was accepted as significant.

Results

In rats receiving continuous infusion colchicine, SDZ PSC-833 had a minimal effect on bile flow (26.1±0.61 μl/min in the 40 min prior to dosing with SDZ PSC-833 and 25.1±0.86 μl/min in the 40 min after treatment with SDZ PSC-833). As shown in Fig. 1A, there was a small and transient decrease in bile flow immediately after bolus injection of SDZ PSC-833. The vehicle, DMSO, was without effect on bile flow except during the last collection. Biliary clearance accounted for approximately 22% of overall systemic clearance. SDZ PSC-833 inhibited colchicine biliary clearance by 76% (basal, 9.05±0.2 ml min^{-1} kg^{-1}; post-SDZ PSC-833, 2.41±0.43 ml min^{-1} kg^{-1}; $P<0.0001$). The inhibition was immediate and lasted for the duration of the experiment. DMSO was without effect on colchicine biliary clearance (Fig. 1B). A bile-to-plasma ratio of greater than 10 is considered indicative of biliary secretion in vivo [9], and the value found for colchicine was 146±8. The colchicine bile-to-plasma ratio was inhibited markedly by SDZ PSC-833 (basal, 146±8; post-SDZ PSC-833, 35±5; $P<0.0001$, Fig. 1C).

Colchicine is also secreted into urine [12]. SDZ PSC-833 seemingly had no effect on the glomerular filtration

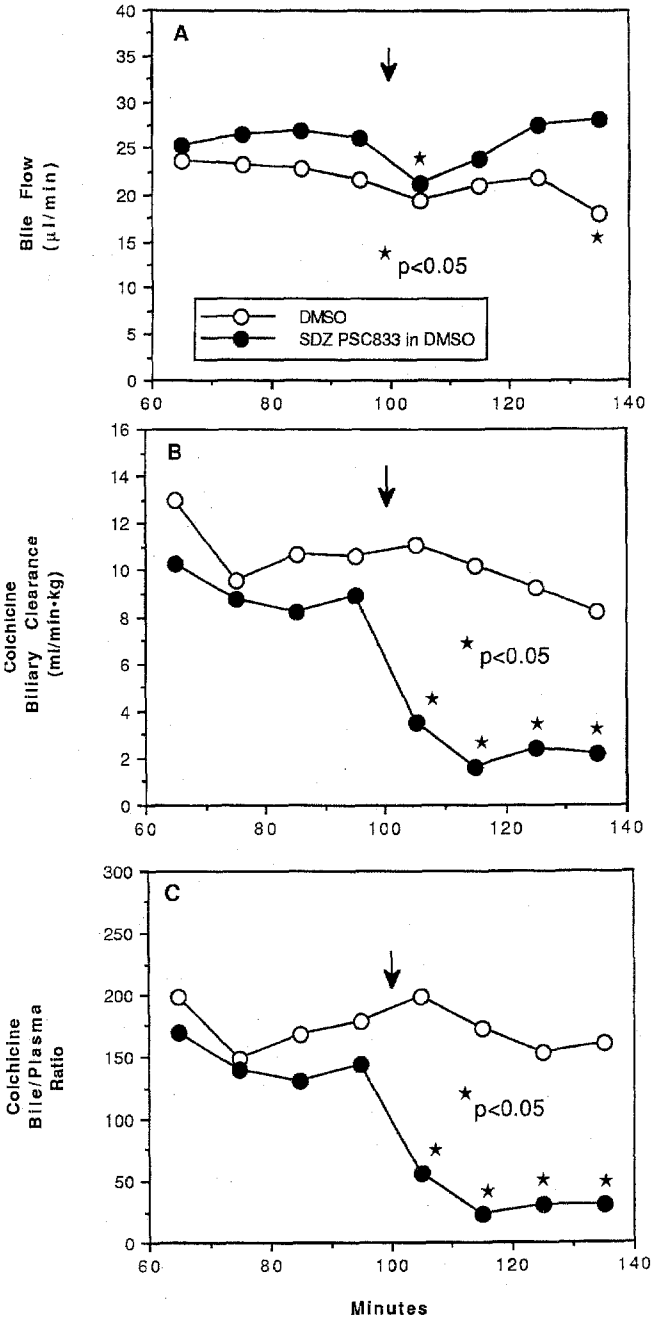

Fig. 1. A–C. Effect of SDZ PSC-833 or vehicle (DMSO) on colchicine biliary secretion in vivo. **A** Bile flow, **B** colchicine biliary clearance, and **C** colchicine bile/plasma ratio in DMSO- (n = 2) and SDZ PSC-833-treated (2 mg/kg, n = 3) rats. Data represent mean values ±SEM. SDZ PSC-833 or DMSO was given as an i.v. bolus at 100 min as indicated by the *arrow*

rate (basal, 3.04±0.24 ml min^{-1} kg^{-1}; post-SDZ PSC-833, 2.881±0.207 ml min^{-1} kg^{-1}; $P>0.5$, Fig. 2A); however, rats treated with DMSO alone had a significantly increased glomerular filtration rate. It is possible that SDZ PSC-833 actually decreases the glomerular filtration rate (difference between the two groups) or blocks the increase secondary to DMSO. The urine flow increased in both groups (basal urine flow, 60.6±3.4 μl/min; post-DMSO, 117.6±4.2 μl/min; P = 0.017; basal urine flow, 49.9±3.9 μl/min; post-SDZ PSC-833, 78.7±10.3 μl/min; P = 0.059). DMSO

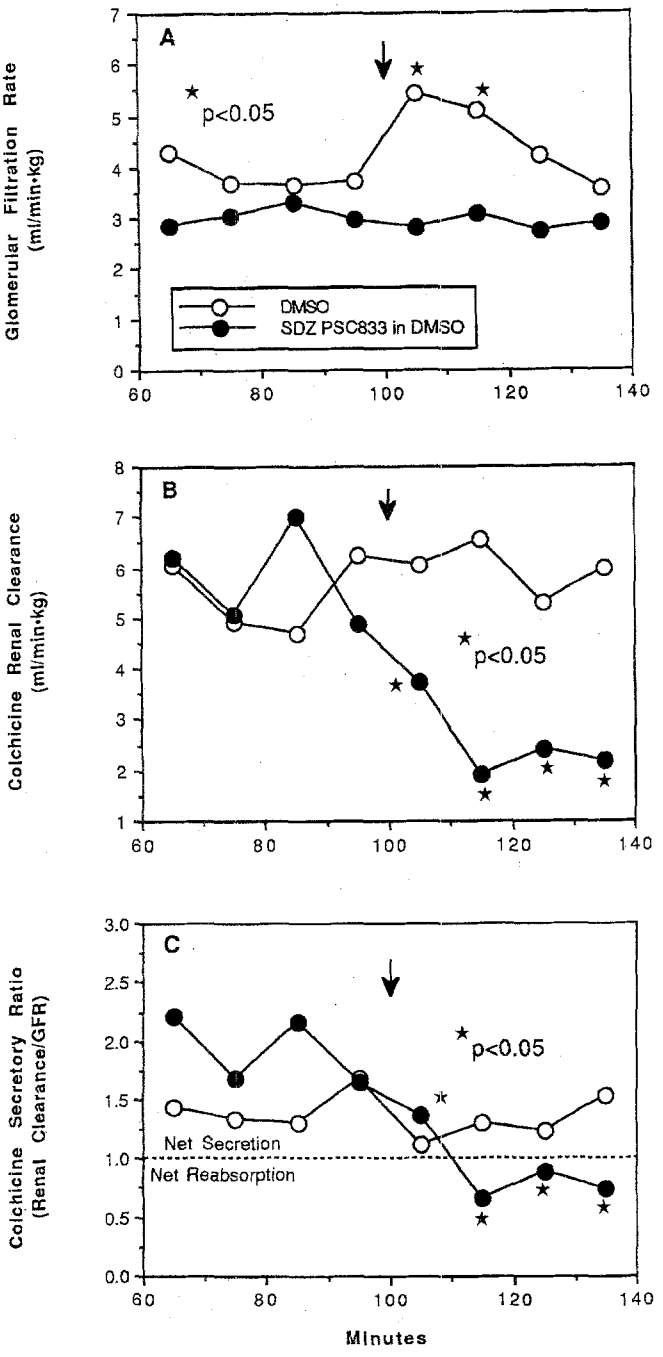

Fig. 2. A–C. Effect of SDZ PSC-833 or vehicle (DMSO) on colchicine renal secretion in vivo. **A** Glomerular filtration rate, **B** colchicine renal clearance, and **C** colchicine secretory ratio (renal clearance/glomerular filtration rate) in DMSO- ($n = 2$) and SDZ PSC-833-treated (2 mg/kg, $n = 3$) rats. Data represent mean values ±SEM. SDZ PSC-833 or DMSO was given as an i.v. bolus at 100 min as indicated by the *arrow*

had no effect on colchicine renal clearance whereas SDZ PSC-833 resulted in a prompt decrease (basal, 6.13 ± 0.34 ml min^{-1} kg^{-1}; post-SDZ PSC-833, 2.57±0.26 ml min^{-1} kg^{-1}; $P = 0.001$, Fig. 2B). Net colchicine renal secretion was abolished by SDZ PSC-833, whereas DMSO was without effect (Fig. 2C).

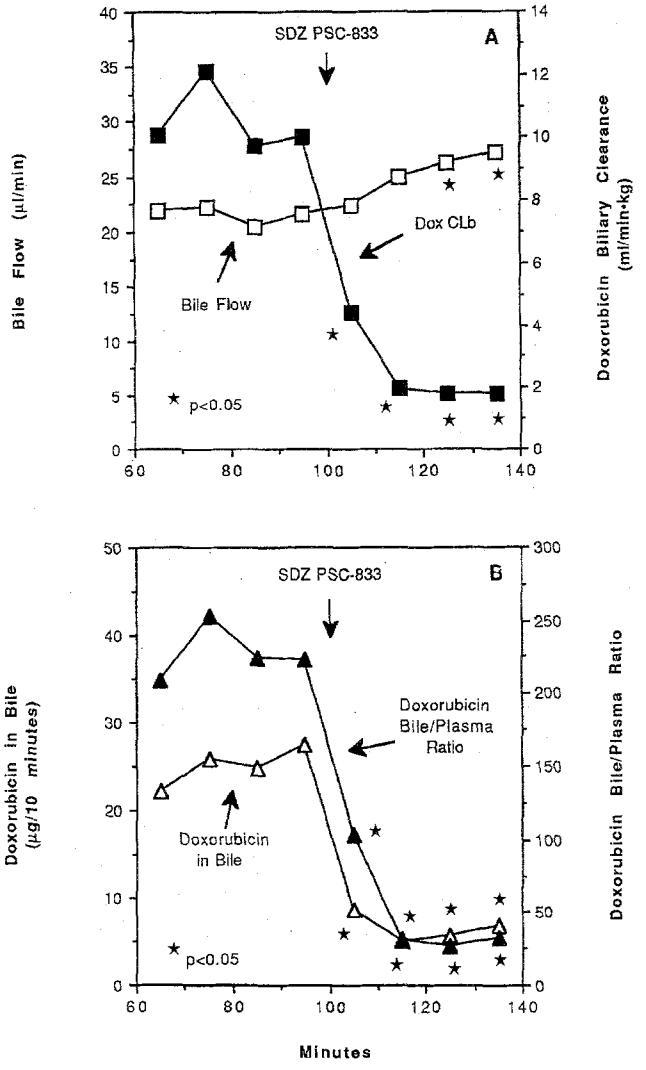

Fig. 3. A., B. Effect of SDZ PSC-833 on doxorubicin biliary secretion in vivo. **A** Bile flow and doxorubicin biliary clearance and **B** doxorubicin in bile for each collection period and doxorubicin bile-to-plasma ratio in SDZ PSC-833-treated (2 mg/kg, $n = 3$) rats. Data represent mean values ±SEM. SDZ PSC-833 was given as an i.v. bolus at 100 min as indicated by the *top arrow*

SDZ PSC-833 resulted in a 63% increase in the average colchicine plasma concentration (basal, 5.69±0.12 µg/ml; post-SDZ PSC-833, 9.25±0.55 µg/ml; $P < 0.01$), whereas DMSO was without effect (basal, 5.44±0.43 µg/ml; post-DMSO, 6.6±0.93 µg/ml; $P = 0.5$). Overall colchicine clearance decreased from 40.66±0.85 to 24.65±1.61 ml min^{-1} kg^{-1} ($P < 0.01$) after SDZ PSC-833 administration.

In rats receiving doxorubicin by continuous infusion, SDZ PSC-833 appeared to cause some choleresis (basal, 21.5±0.58 µl/min; post-SDZ PSC-833, 25.2±1.17 µl/min; $P = 0.01$), but this was accounted for by the last collections (Fig. 3A). SDZ PSC-833 markedly inhibited doxorubicin biliary clearance (basal, 10.5±1.36 ml min^{-1} kg^{-1}; post-SDZ PSC-833, 2.48±0.64 ml min^{-1} kg^{-1}; $P = 0.0001$). The amount of doxorubicin appearing in bile decreased (Fig. 3B). Doxorubicin is extensively secreted into bile with the bile-to-plasma ratio being 228±28. SDZ PSC-833

markedly inhibited such secretion, decreasing the bile-to-plasma ratio by 79% (post-SDZ PSC-833, 48 ± 18; $P < 0.01$, Fig. 3C). The plasma concentration of doxorubicin increased from 0.63 ± 0.21 to 0.83 ± 0.27 μg/ml after SDZ PSC-833 administration ($P = 0.08$).

Discussion

This study demonstrates that the nonimmunosuppressive cyclosporin D analog SDZ PSC-833 is capable of markedly inhibiting the biliary secretion of two cytotoxic agents known to be substrates for P-glycoprotein, namely, colchicine and doxorubicin. It is presumably the P-glycoprotein in the hepatocyte canaliculus [5, 14, 15] that is being inhibited. Because the inhibition is rapid and long lasting, it is assumed that SDZ PSC-833 is readily taken up by the liver, although this was not studied. SDZ PSC-833 appears to be at least as potent as cyclosporin as an inhibitor of colchicine biliary secretion. In a previous study using a similar technique, cyclosporin given as a 2-mg/kg bolus injection resulted in a 64% decrease in colchicine biliary clearance and a 10-mg/kg bolus resulted in an 81% decrease in colchicine biliary clearance [11] as compared with the 76% decrease obtained with a 2-mg/kg bolus of SDZ PSC-833 in the present study. The colchicine bile-to-plasma ratio decreased by 64% following a 2-mg/kg dose of cyclosporin and decreased by 77% following a 10-mg/kg dose of cyclosporin as compared with the 76% decrease observed following SDZ PSC-833 administration. In the previous study cyclosporin was given in cremophor, which also has an effect on both colchicine biliary clearance and the colchicine bile-to-plasma ratio [11].

Colchicine secretion into urine occurs by a process separate from the organic anion or organic cation transporters in the kidney tubule [12] and is presumably a consequence of the P-glycoprotein located in the kidney proximal tubule [14, 15]. This study also demonstrates that SDZ PSC-833 inhibits the renal secretion of colchicine. In other studies we have found that doxorubicin is also secreted into rat urine; however, for unknown reasons, two of the three rats receiving doxorubicin in these experiments had no demonstrable net secretion. In the third rat which did have doxorubicin secretion, SDZ PSC-833 markedly inhibited the drug's renal secretion.

As has been pointed out by other investigators, SDZ PSC-833 appears to be a promising chemosensitizer and may allow a test of the hypothesis that P-glycoprotein contributes to the failure of chemotherapy in vivo and that reversal of multidrug resistance will be a useful clinical strategy [17]. It would appear that chemosensitizers such as SDZ PSC-833 and cyclosporin are capable of impairing the normal elimination of drugs such as colchicine and doxorubicin, which depend on biliary and renal secretion as pathways of normal elimination. Such an impairment may lead to increased toxicity to normal tissues.

References

1. Boekhorst PAW te , Kapel J van, Schoester M, Sonneveld P (1992) Reversal of typical multidrug resistance by cyclosporin and its non-immunosuppressive analogue SDZ PSC-833 in Chinese hamster ovary cells expressing the mdr1 phenotype. Cancer Chemother Pharmacol 30: 238
2. Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loor F (1991) In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC-833. Cancer Res 51: 4226
3. Friche E, Jensen PB, Nissen NI (1992) Comparison of cyclosporin A and SDZ PSC-833 as multidrug-resistance modulators in a daunorubicin-resistant Ehrlich ascites tumor. Cancer Chemother Pharmacol 30: 235
4. Heyrovsky A (1956) A new method for the determination of inulin in plasma and urine. Clin Chim Acta 1: 470
5. Kamimoto Y, Gatmaitan Z, Hsu J, Ariar IM (1989) The function of Gp170, the multidrug resistance gene product, in rat liver canalicular membrane vesicles. J Biol Chem 264: 11693
6. Keller RP, Altermatt HJ, Nooter K, Poschmann G, Laissue JA, Bollinger P, Hiestand PC (1992) SDZ PSC 833, a non-immunosuppressive cyclosporin: its potency in overcoming P-glycoprotein-mediated multidrug resistance of murine leukemia. Int J Cancer 50: 593
7. Nielsen D, Skovsgaard T (1992) P-glycoprotein as multidrug transporter: a critical review of current multidrug resistant cell lines. Biochim Biophys Acta 1139: 169
8. Riggs CE, Benjamin RS, Serpick AA, Bachur NR (1977) Biliary disposition of Adriamycin. Clin Pharmacol Ther 22: 234
9. Schanker LS (1968) Secretion of organic compounds in bile. In: Code CF, Heidel W (eds) Handbook of physiology, section 6: alimentary canal. American Physiological Society, Washington, D.C., p 2433
10. Slater LM, Sweet P, Stupeky M, Gaptz S (1986) Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. J Clin Invest 77: 1405
11. Speeg KV, Maldonado AL, Liaci J, Muirhead D (1992) Effect of cyclosporin on colchicine secretion by a liver canalicular transporter studied in vivo. Hepatology 15: 899
12. Speeg KV, Maldonado AL, Liaci J, Muirhead D (1992) Effect of cyclosporin on colchicine secretion by the kidney multidrug transporter studied in vivo. J Pharmacol Exp Ther 261: 50
13. Sugawara I, Kataoka I, Morishita Y, Hamada H, Tsuruo T, Itoyama S, Mori S (1988) Tissue distribution of P-glycoprotein encoded by a multidrug-resistance gene as revealed by a monoclonal antibody, MRK 16. Cancer Res 48: 1926
14. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 84: 7735
15. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1989) Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: evidence for localization in brain capillaries and crossreactivity of one antibody with a muscle protein. J Histochem Cytochem 37: 159
16. Twentyman PR (1988) Modification of cytotoxic drug resistance by non-immunosuppressive cyclosporin. Br J Cancer 57: 254
17. Twentyman PR (1992) Cyclosporins as drug resistance modifiers. Biochem Pharmacol 43: 109