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Chronic cyclosporine administration induces renal P-glycoprotein in rats

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Abstract

The effect of cyclosporine doses on renal P-glycoprotein expression was examined. Rats were given cyclosporine orally at 2, 10, 30 mg/kg/day or subcutaneously at 1, 5, 15 mg/kg/day for 28 days with or without 14 days of additional vehicle dosing. Following cyclosporine dosing, renal function and P-glycoprotein expression were measured. Renal function was reduced in rats receiving oral cyclosporine and the highest subcutaneous dose, 15 mg/kg/day. Western blot analysis showed that cyclosporine administered orally at 10 and 30 mg/kg/day and subcutaneously at 15 mg/kg/day induced significantly renal P-glycoprotein expression. After discontinuation of cyclosporine, renal P-glycoprotein returned to pre-dosing levels in oral groups, whereas the return was incomplete in subcutaneous groups. These results indicate that cyclosporine induces renal P-glycoprotein overexpression a dose-dependent manner.

Keywords: Cyclosporine; P-glycoprotein, mdr1; Renal; Nephrotoxicity

1. Introduction

Cyclosporine is an immunosuppressant widely used for the prevention of immune rejection of transplanted tissues and graft-versus-host disease after bone marrow transplantation. Although cyclosporine has been used with considerable success, it has been shown to be nephrotoxic in clinical (Myers, 1986) and experimental studies (Rossi et al., 1989). This nephrotoxicity is characterized by acute and chronic phases (Bennett and Pulliam, 1983) and is the major dose-limiting factor in the use of the drug. Acute toxicity is reversible and is associated with reduction in glomerular filtration and creatinine clearance, an increase in proximal tubular reabsorption, and elevated serum creatinine and urea nitrogen levels (Bennett and Pulliam, 1983; Keown et al., 1986). Progression from acute to chronic toxicity leads to irreversible nephrotoxicity characterized by tubulointerstitial fibrosis and glomerulosclerosis attributed to enhanced accumulation of extracellular matrix components (Kopp and Klotman, 1990).

The mechanism of cyclosporine nephrotoxicity remains unclear and more than one factor may be involved. The high affinity of cyclosporine for P-glycoprotein (Foxwell et al., 1989; Rao and Scarborough, 1994) suggests that P-glycoprotein may be one of the factors involved in cyclosporine-induced nephrotoxicity. P-glycoprotein is a phosphorylated and glycosylated membrane protein (Gottesman and Pastan, 1993) that was first discovered by its ability to confer multidrug resistance to cancer cells by functioning as an efflux pump (Biedler and Riehm, 1970).

At present, humans are known to have only one gene, *MDR*1, encoding P-glycoprotein (Chin et al., 1989). In contrast, rodents have two genes encoding P-glycoprotein (mdr1a and mdr1b), which together fulfill the same function as *MDR*1 in humans (Devault and Gros, 1990; Gros et al., 1986). P-glycoprotein is found in the apical membrane of polarized transporting epithelial cells in the liver, intestine, and kidney (Thiebaut et al., 1987). These distributions suggest that P-glycoprotein is involved with protecting the organism against xenobiotic toxicity.

P-glycoprotein is constitutively expressed in the brush border of proximal convoluted tubules (Ernest and Bello-Reuss, 1995; Lieberman et al., 1989), the mesangial cells (Bello-Reuss and Ernest, 1994), the thick ascending limb of Henle's loop as well as the collecting duct of the kidney (Ernest and Bello-Reuss, 1998). Because renal P-glycoprotein interacts with several structurally unrelated compounds (Charuk et al., 1994, 1995), it is likely that P-glycoprotein participates in the excretion of xenobiotics and endogenous toxic metabolites into the urine and plays a role in drug-associated nephrotoxicity.

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The purpose of the present study was to examine the expression of renal P-glycoprotein following chronic cyclosporine therapy by different administration routes and at different dosing levels. These data will provide information regarding how P-glycoprotein may be involved with the complex mechanisms associated with cyclosporine-induced nephrotoxicity.

2. Materials and methods

2.1. Materials

Cyclosporine oral solution was generously provided by Novartis Pharmaceuticals (East Hanover, NJ). The vehicle was a solution of 12.5% vol/vol ethanol in olive oil. Furosemide was purchased from American Reagent Laboratories, (Shirley, NY). Other reagents used for gel electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA). Leupeptin, aprotinin and phenylmethylsulfonyl fluoride were purchased from Sigma (St. Louis, MO). Mouse anti-rat monoclonal antibody C219 was purchased from Signet Laboratories (Dedham, MA).

2.2. Animals

Ten-week-old male Sprague–Dawley rats ($N = 110$) were purchased from Harlan Sprague–Dawley (Indianapolis, IN) and individually housed in wire-bottom cages in a 12-h light/dark cycle animal facility with controlled humidity and temperature. Approval of the protocol was given by The University of Texas at Austin Animal Care and Use Committee prior to the start of the study.

2.3. Experimental design

Following a 5-day acclimation period, rats were given a single intraperitoneal dose of furosemide (4 mg/kg) to motivate initiation of salt depletion and started on a low-salt rice diet as previously described (Brunner et al., 1998). Depending on the routes of administration, rats were randomly allocated into four experimental groups, each of which had four subgroups ($N = 6–8$ each group). Rats in Group 1 were given cyclosporine orally at doses of 2, 10, or 30 mg/kg/day or 1 ml/kg/day olive oil vehicle for 28 days. Rats in Group 2 were also given cyclosporine orally at the same doses as Group 1 for 28 days, but cyclosporine dosing was stopped at day 29, and 1 ml/kg/day olive oil vehicle was given for an additional 14 days. Rats in Group 3 were subcutaneously injected with cyclosporine at doses of 1, 5, or 15 mg/kg/day or 1 ml/kg/day olive oil vehicle for 28 days. Rats in Group 4 were also injected with cyclosporine at the same doses as Group 3 for 28 days, but cyclosporine dosing was stopped at day 29 and 1 ml/kg/day vehicle was given for an additional 14 days. Cyclosporine doses were chosen based on our previous work studies with this rat model (Brunner et al., 1996, 1998, 2000). To minimize chronobiologic variability in drug toxicity, cyclosporine and vehicle were administered at the same time each day to the rats (Luke et al., 1988). Body weights and food intake were measured daily. On the last day of drug treatment or vehicle dosing, the rats were placed into standard rodent metabolic cages (Nalge, Rochester, NY) for passive urine collection for 24 h. After the urine collection period, the rats were anesthetized with a single dose of 1 ml/kg of an anesthetic mixture (ketamine 100 mg/ml, xylazine 20 mg/ml, and acepromazine 10 mg/ml at a volume ratio of 1:1:1). The rat kidneys were removed, immediately frozen in liquid nitrogen, and then stored at −80°C.

2.4. Microsome isolation

The rat renal microsomes were isolated by differential centrifugation as previously described (Brunner et al., 1998). Samples were maintained at 4°C during the microsome preparation. In brief, whole rat kidneys were homogenized with a tissue homogenizer in 3 ml of Tris–HCl buffer, pH 7.4, containing 150 mM potassium chloride and 1 mM EDTA, 5 μg/ml leupeptin, 10 μg/ml aprotinin, and 100 μM phenylmethylsulfonyl fluoride. The samples were then centrifuged at $9000 \times g$ for 20 min at 4°C. The supernatant was collected and centrifuged at $490{,}000 \times g$ for 17 min. The supernatant was then discarded, and the pellet was resuspended with a tissue grinder and washed in a sodium pyrophosphate buffer, pH 7.4, containing 1 mM EDTA. The suspension was again centrifuged again at $490{,}000 \times g$ for 17 min at 4°C. The supernatant was discard, and the washed pellet was resuspended with the tissue grinder in a Tris–HCl buffer, pH 7.4, containing 20% glycerol. Renal microsomes were stored at −80°C prior to analysis.

2.5. Gel electrophoresis and immunoblot analysis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously (Laemmli et al., 1970) with l50-μg renal microsomes loaded per well on an 8% polyacrylamide separating gel. After separation, proteins were transferred electrophoretically to nitrocellulose sheets with an electrophoretic blotter as described previously (Schnier et al., 1989). For the detection of P-glycoprotein immunoreactive proteins, nitrocellulose sheets were blocked with 5% nonfat dried milk (NFDM) in Tris-buffered saline (TBS) for 1 h and incubated with a 1:1000 dilution of mouse anti-rat monoclonal antibody C219 (in 1% NFDM in TBS) overnight, followed by a 1:2000 dilution of rat anti-mouse IgG (in 5% NFDM in TBS) for

1 h. All incubations were performed at ambient temperature. Immune complexes for P-glycoprotein immunoreactive bands were detected with a chemiluminescence reagent (NEN™ Life Sciences Products, Boston, MA).

2.6. Other assays

Microsomal protein concentrations were determined by standard methods using bovine serum albumin as a reference (Lowry et al., 1951). Urine and serum creatinine were measured using the modified Jaffe reaction (Sigma Diagnostics, St. Louis, MO).

2.7. Statistical analysis

Differences between rat groups were compared using one-factor analysis of variance with Bonferroni/Dunn post-hoc analysis (SuperANOVA; Abacus Concepts, Berkeley, CA). Data are presented as means ± S.E. Differences were considered significant when the probability of chance explaining the results was reduced to less than 5% ($P < 0.05$).

3. Results

All the rats gained weight over the study period, and there was no significant effect of drug treatment or length of treatment on weight gain. Table 1 is a summary of the effect of cyclosporine treatment on markers of renal function. Urine output over a 24-h period was significantly greater in rats treated orally with cyclosporine at doses of 10 mg/kg ($P < 0.01$) and 30 mg/kg ($P < 0.001$). Serum creatinine was also elevated significantly in these two groups. Creatinine clearance was decreased in rats given 2, 10, or 30 mg/kg cyclosporine, indicating a significant reduction of renal function during chronic cyclosporine dosing. However, renal function recovered to control levels 14 days after the discontinuation of cyclosporine dosing.

Rats administered cyclosporine by subcutaneous injection also showed alterations in renal function. The 24-h urine volume was significantly greater in rats treated with 15 mg/kg cyclosporine ($P < 0.001$) than in the vehicle group. The cyclosporine group also showed a corresponding increase in serum creatinine ($P < 0.01$) and a significant reduction in creatinine clearance ($P < 0.05$). However, after 14 additional days of vehicle dosing, renal functional parameters did not fully return to control values.

To examine the expression of renal P-glycoprotein, immunoblot analysis of renal microsomal protein was performed using a mouse anti-rat monoclonal antibody. Renal microsomal protein from rats treated with olive oil or with varying doses of cyclosporine showed immunoreactive bands corresponding with standard bands of human membrane-associated P-glycoprotein (Fig. 1A). Renal P-glycoprotein expression in rats orally treated with cyclosporine is shown in Fig. 1B. Following 28 days of treatment, renal P-glycoprotein expression significantly increased by 139% ($P < 0.05$), 145% ($P < 0.05$) and 164% ($P < 0.01$) in rats treated orally with cyclosporine at 2, 10, and 30 mg/kg, respectively. Following 14 days of vehicle dosing after the cessation of cyclosporine treatment, renal P-glycoprotein levels recovered to control values.

After 28 days of cyclosporine treatment, renal P-glycoprotein significantly increased by 162% ($P < 0.05$) and 200% ($P < 0.01$) in rats injected subcutaneously with 5 and 15 mg/kg cyclosporine, respectively (Fig. 1C). However, renal P-glycoprotein expression in rats given cyclosporine 1 mg/kg did not change significantly. Discontinuation of cyclosporine caused P-glycoprotein levels to return to pretreatment levels in rats receiving 5 mg/kg cyclosporine after 14 days of vehicle dosing. However, the

Table 1
The effect of cyclosporine (CsA) on markers of renal function with oral administration of 2, 10 and 30 mg/kg and subcutaneous injection of 1, 5 and 15 mg/kg for 28 days and for an additional 14 days of vehicle dosing

Group (Oral)	28 Days				42 Days			
	Vehicle	CsA2	CsA10	CsA30	Vehicle	CsA2	CsA10	CsA30
U Vol.	9 ± 1	7 ± 2	21 ± 3[a]	26 ± 3[b]	8 ± 2	10 ± 2	12 ± 2	12 ± 2
Scr	0.44 ± 0.03	0.6 ± 0.03	0.73 ± 0.07[a]	0.85 ± 0.06[b]	0.44 ± 0.03	0.49 ± 0.06	0.44 ± 0.03	0.55 ± 0.06
CrCl	460 ± 38	345 ± 46[c]	291 ± 42[a]	230 ± 28[b]	419 ± 48	400 ± 53	425 ± 35	308 ± 40
Group (Subcutaneous)	Vehicle	CsA1	CsA5	CsA15	Vehicle	CsA1	CsA5	CsA15
U Vol.	7 ± 1	10 ± 1	13 ± 2	24 ± 4[b]	12 ± 1	12 ± 2	14 ± 1	20 ± 2[c]
Scr	0.43 ± 0.03	0.46 ± 0.03	0.53 ± 0.06	0.72 ± 0.05[a]	0.46 ± 0.04	0.46 ± 0.04	0.48 ± 0.08	0.68 ± 0.08
CrCl	440 ± 63	467 ± 34	410 ± 41	298 ± 20[c]	467 ± 31	452 ± 54	427 ± 69	337 ± 34[c]

U Vol., Urine volume (ml); Scr, Serum creatinine (mg/dl); CrCl, Creatinine clearance (μl/min/100 g).
[a] $P < 0.01$ as compared with vehicle group.
[b] $P < 0.001$ as compared with vehicle group.
[c] $P < 0.05$ as compared with vehicle group.

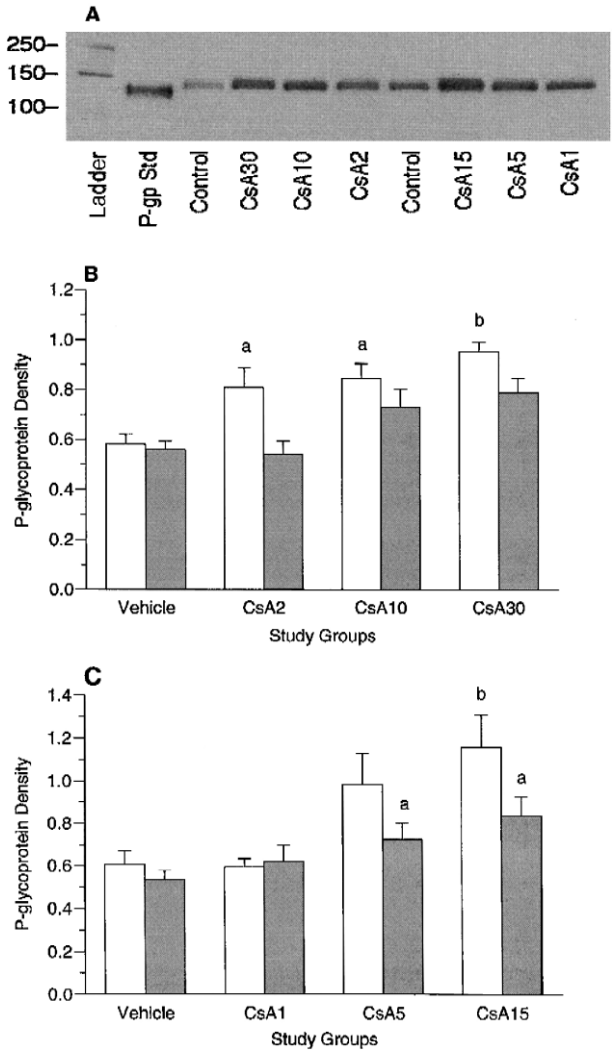

Fig. 1. P-glycoprotein (P-gp) expression in the rat renal microsomes. The immunoblot was probed with mouse anti-rat monoclonal antibody C219 (A). Quantification of P-glycoprotein expression in rats treated for 28 days (open bars) of once daily oral cyclosporine dosing (B) at 2 mg/kg (CsA2), 10 mg/kg (CsA10), 30 mg/kg (CsA30) or subcutaneous injections (C) at 1 mg/kg (CsA1), 5 mg/kg (CsA5), and 15 mg/kg (CsA15) or with an additional 14 days of 1 ml/kg olive oil vehicle dosing (filled bars). $^{a}P < 0.05$ and $^{b}P < 0.01$ as compared with treatment-matched vehicle controls.

return of renal P-glycoprotein expression in rats injected with 15 mg/kg was incomplete.

4. Discussion

In our present study, rats receiving chronic cyclosporine treatment orally at doses of 10 and 30 mg/kg or subcutaneously at 15 mg/kg developed significant nephrotoxicity with increases in serum creatinine and corresponding decreases in creatinine clearance. Although serum creatinine in the rats treated with 2 mg/kg cyclosporine increased as compared with controls, the increase was not statistically significant. After cessation of cyclosporine administration and an additional 14 days of vehicle treatment, serum creatinine and creatinine clearance recovered to control levels in orally treated groups, whereas the return was not complete in the subcutaneously injected groups. Thus, the functional change produced by cyclosporine in this rat model appears to be reversible after removal of the drug, but the time for recovery is dosing route-dependent.

In addition to immunosuppression, cyclosporine has also been used as a drug modifier to restore the sensitivity of cancerous cells to chemotherapeutic agents (Twentyman, 1992). This mechanism is based on its competitive inhibition with substrates to transporter P-glycoprotein (Foxwell et al., 1989; Goldberg et al., 1988; Tamai and Safa, 1990). Bennett et al. (1996) recently proposed that cyclosporine may act as a P-glycoprotein blocker in the renal tubule via a mechanism of competitive inhibition that may lead to the accumulation of an endogenous toxin or of toxic cyclosporine metabolites in tubular cells. Many in vitro and in vivo investigations demonstrated that cyclosporine induces renal P-glycoprotein expression. Hauser et al. (1998) demonstrated that therapeutic concentrations of cyclosporine increase P-glycoprotein expression in renal tubule cells and endothelial cells. Furthermore, work by Garcia del Moral et al. (1995) showed that exposure of the Madin–Darby canine kidney (MDCK) dog renal epithelial cell line to cyclosporine increases the expression of P-glycoprotein.

We examined renal microsomes in the current study since the P-glycoprotein is expressed not only in the proximal tubule cells (Thiebaut et al., 1987), but also in the plasma membrane of mesangial cells, the apical membrane of the thick ascending limb of Henle's loop, and the apical membrane of the collecting duct as well (Ernest et al., 1997) Although the thick ascending limb of Henle's loop and the collecting duct are the segments of the nephron that are not traditionally involved in the excretion of xenobiotics, P-glycoprotein may transport metabolites produced endogenously by those cells, or could also prevent the uptake of substances entering the lumen (Ernest and Bello-Reuss, 1998).

This in vivo study also demonstrated that rats treated with cyclosporine developed chronic nephrotoxicity lesions, and overexpression of P-glycoprotein in the renal tubules showed a strong inverse relationship with the incidence of hyaline arteriopathy and periglomerular and peritubular fibrosis (Del Moral et al., 1997). Furthermore, patients with no evidence of positive P-glycoprotein induction had the highest incidence of nephrotoxic lesions (Garcia del Moral et al., 1995). In addition, upregulation of mdr1b was demonstrated in mdr1a $-/-$ knockout mice compared to wild-type mice. This upregulation may be due to a compensatory response of the kidney to the absence of mdr1a, resulting in increased concentrations of endogenous substrates (Schinkel et al., 1994).

In the present study, rats were given escalating doses of cyclosporine via oral or subcutaneous routes. Renal microsomes for renal P-glycoprotein measurement were used based on that P-glycoprotein is present throughout the kidney (Ernest and Bello-Reuss, 1998). Renal P-glycoprotein expression was significantly greater in rats orally treated with 2, 10, or 30 mg/kg cyclosporine or subcutaneously injected with 5 or 15 mg/kg cyclosporine than in treatment-matched controls. In addition, after discontinuation of cyclosporine treatment following the additional 14 days of vehicle dosing, renal P-glycoprotein expression in oral groups returned to control levels. These findings indicated that the induction of renal P-glycoprotein was a reversible process and was not dependent on the route of cyclosporine administration. In addition, our data also showed that the time required for renal P-glycoprotein levels to return to pretreatment levels in the group injected subcutaneously was longer than in the group treated orally since the absorption process in the subcutaneous route may be delayed relative to the oral route. Taken together, these in vitro and in vivo findings support the hypothesis that renal P-glycoprotein overexpression may play a favorable role in protection against cyclosporine-induced nephrotoxicity by serving to excrete xenotoxins or metabolites from the body.

In conclusion, our results show that chronic cyclosporine treatment reversibly induces renal P-glycoprotein expression and that this induction occurs in a dose-dependent manner and is independent of administration routes. In addition, these results implicate the potential clinical difficulty of using cyclosporine as a modifier for reversing multidrug resistance and drug interactions involving renal excretory mechanisms. These data help increase the understanding of how chronic administration of cyclosporine alters renal P-glycoprotein and support the concept of P-glycoprotein functioning as efflux pump protecting against cyclosporine-induced nephrotoxicity.

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References

Bello-Reuss, E., Ernest, S., 1994. Expression and function of P-glycoprotein in human mesangial cells. Am. J. Physiol. 267, C1351–C1358.

Bennett, W.M., Pulliam, J.P., 1983. Cyclosporine nephrotoxicity. Ann. Intern. Med. 99, 851–854.

Bennett, W.M., DeMattos, A., Meyer, M.M., Andoh, T., Barry, J.M., 1996. Chronic cyclosporine nephropathy: the Achilles' heel of immunosuppressive therapy. Kidney Int. 50, 1089–1100.

Biedler, J.L., Riehm, H., 1970. Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic, and cytogenetic studies. Cancer Res. 30, 1174–1184.

Brunner, L.J., Bennett, W.M., Koop, D.R., 1996. Selective suppression of rat hepatic microsomal activity during chronic cyclosporine nephrotoxicity. J. Pharmacol. Exp. Ther. 277, 1710–1718.

Brunner, L.J., Bennett, W.M., Koop, D.R., 1998. Cyclosporine suppresses rat hepatic cytochrome P450 in a time-dependent manner. Kidney Int. 54, 216–223.

Brunner, L.J., Werner, U., Gravenall, C.E., 2000. Effect of dose on cyclosporine-induced suppression of hepatic cytochrome P450. Eur. J. Pharm. Biopharm. 49, 129–135.

Charuk, J.H., Loo, T.W., Clarke, D.M., Reithmeier, R.A., 1994. Interaction of rat kidney P-glycoprotein with a urinary component and various drugs including cyclosporine A. Am. J. Physiol. 266, F66–F751, Pt 2.

Charuk, J.H., Wong, P.Y., Reithmeier, R.A., 1995. Differential interaction of human renal P-glycoprotein with various metabolites and analogues of cyclosporine A. Am. J. Physiol. 269, F31–F391, Pt 2.

Chin, J.E., Soffir, R., Noonan, K.E., Choi, K., Roninson, I.B., 1989. Structure and expression of the human *MDR* (P-glycoprotein) gene family. Mol. Cell. Biol. 9, 3808–3820.

Del Moral, R.G., Andujar, M., Ramirez, C., Gomez-Morales, M., Masseroli, M., Aguilar, M., Olmo, A., Arrebola, F., Guillen, M., Garcia-Chicano, M.J., Nogales, F.F., O'Valle, F., 1997. Chronic cyclosporine A nephrotoxicity, P-glycoprotein overexpression, and relationships with intrarenal angiotensin II deposits. Am. J. Pathol. 151, 1705–1714.

Devault, A., Gros, P., 1990. Two members of mouse mdr gene family confer multidrug resistance with overlapping but distinct drug specificities. Mol. Cell. Biol. 10, 1652–1663.

Ernest, S., Bello-Reuss, E., 1995. Expression and function of P-glycoprotein in a mouse kidney cell line. Am. J. Physiol. 269, C323–C333.

Ernest, S., Bello-Reuss, E., 1998. P-glycoprotein functions and substrates: possible roles of *MDR*1 gene in the kidney. Kidney Int. 53, S11–S17.

Ernest, S., Rajaraman, S., Megyesi, J., Bello-Reuss, E.N., 1997. Expression of *MDR*1 (Multidrug resistance) gene and its protein in normal human kidney. Nephron 77, 284–289.

Foxwell, B.M., Mackie, A., Ling, V., Ryffel, B., 1989. Identification of the multidrug resistance-related P-glycoprotein as a cyclosporine binding protein. Mol. Pharmacol. 36, 543–546.

Garcia del Moral, R., O'Valle, F., Andujar, M., Aguilar, M., Lucena, M.A., Lopez-Hidalgo, J., Ramirez, C., Medina-Cano, M.T., Aguilar, D., Gomez-Morales, M., 1995. Relationship between P-glycoprotein expression and cyclosporine A in kidney: an immunohistological and cell culture study. Am. J. Pathol. 146, 398–408.

Goldberg, H., Ling, V., Wong, P.Y., Skorecki, K., 1988. Reduced cyclosporine accumulation in multidrug-resistant cells. Biochem. Biophys. Res. Commun. 152, 552–558.

Gottesman, M.M., Pastan, I., 1993. Biochemistry of multidrug resistance mediated by multidrug transporter. Annu. Rev. Biochem. 62, 385–427.

Gros, P., Croop, J., Housman, D., 1986. Mammalian multidrug resistance gene: complete cDNA sequence indicates strong homology to bacterial transport protein. Cell 47, 371–380.

Hauser, I.A., Koziolek, M., Hopfer, U., Thevenod, F., 1998. Therapeutic concentrations of cyclosporine A, but not FK506, increase P-glycoprotein expression in endothelial and renal tubule cells. Kidney Int. 54, 1139–1149.

Keown, P.A., Stiller, C.R., Wallace, A.C., 1986. The nephrotoxicity of cyclosporine. Marcel Dekker, New York, NY.

Kopp, J.B., Klotman, P.E., 1990. Cellar and molecular mechanism of cyclosporine nephrotoxicity. J. Am. Soc. Nephrol. 6, 162–179.

Laemmli, U.K., Molbert, E., Showe, M., Kellenberger, E., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lieberman, D.M., Reithmeier, R.A., Ling, V., Charuk, J.H., Goldberg, H., Skorecki, K.L., 1989. Identification of P-glycoprotein in renal brush border membranes. Biochem. Biophys. Res. Commun. 162, 244–252.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luke, D.R., Vadiei, K., Brunner, L.J., 1988. Time-dependent pharmacokinetics and toxicity of cyclosporine. Chronobiol. Int. 5, 353–362.

Myers, B.D., 1986. Cyclosporine nephrotoxicity. Kidney Int. 30, 964–974.

Rao, U.S., Scarborough, G.A., 1994. Direct demonstration of high affinity interactions of immunosuppressant drugs with the drug binding site of the human P-glycoprotein. Mol. Pharmacol. 45, 773–776.

Rossi, N.F., Churchill, P.C., McDonald, F.D., Ellis, V.R., 1989. Mechanism of cyclosporine A-induced renal vasoconstriction in the rat. J. Pharmacol. Exp. Ther. 250, 896–901.

Schinkel, A.H., Smit, J.J., van Telligen, O., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol, C.A., van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P., Berns, A.J.M., Borst, P., 1994. Disruption of the mouse mdr 1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. Cell 77, 491–502.

Schnier, G.G., Laethem, C.L., Koop, D.R., 1989. Identification and induction of cytochromes *P*450, *P*450IIE1, and *P*450 IA1 in rabbit bone marrow. J. Pharmacol. Exp. Ther. 251, 790–796.

Tamai, I., Safa, A.R., 1990. Competitive interaction of cyclosporine with the vinca alkaloid-binding site of P-glycoprotein in multidrug-resistant cells. J. Biol. Chem. 265, 16509–16513.

Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I., Willingham, M.C., 1987. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc. Natl. Acad. Sci. U. S. A., 7735–7738.

Twentyman, P.R., 1992. Cyclosporine as drug resistance modifiers. Biochem. Pharmacol. 43, 109–117.