0022-3565/94/2691-0022$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 269, No. 1
Printed in U.S.A.

Cyclosporin A Markedly Changes the Distribution of Doxorubicin in Mice and Rats[1]

TINA COLOMBO, MASSIMO ZUCCHETTI and MAURIZIO D'INCALCI
Laboratory of Cancer Chemotherapy, Istituto di Ricerche Farmacologiche, "Mario Negri," Milan, Italy
Accepted for publication November 11, 1993

ABSTRACT

Cyclosporin A (CsA) inhibits the membrane transport protein Pgp and can thus restore sensitivity to doxorubicin (Dx) and other antineoplastic agents in multidrug resistant cancer cells. Because Pgp is not expressed only in resistant tumor cells but also in normal tissues, CsA may modify the distribution of a concomitantly given antitumor agent which is a substrate for Pgp-mediated transport. We investigated Dx distribution in rats and mice injected i.p. with CsA then 30 min later with an i.v. dose of Dx. In both species CsA treatment markedly increased Dx concentrations in liver, adrenals and kidney, with a small but significant increase in the heart and no change in brain. The CsA-induced changes in Dx tissue levels were not related to inhibition of Dx metabolism or reduced fecal and renal Dx elimination. A marked delayed lethal toxicity of Dx was seen in mice treated with CsA and Dx but not with either drug alone. These results indicate that CsA can profoundly alter Dx pharmacokinetics and toxicity and suggest that caution is advisable when prescribing this combination to cancer patients using full Dx doses.

The MDR mechanism is the best characterized mechanism of tumor cell drug resistance so far identified. It involves the expression of a glycoprotein of 170 kDa molecular weight (Pgp), which acts as an energy-dependent pump able to increase the efflux, and reduce the intracellular retention of several lipophilic cytotoxic agents (Gottesman, 1993). Pgp is involved in a number of tumor cell lines of human or rodent origin which are resistant to many of the widely used anticancer agents including anthracyclines, vinca alkaloids, podophyllotoxins, actinomycin D and taxol (see Endicott and Ling, 1989; van der Bliek and Borst, 1989, as reviews).

Although most data have been obtained *in vitro*, in either drug-resistant cell lines (Juliano and Ling, 1976; Beck *et al.*, 1979; Riordan *et al.*, 1985; Fojo *et al.*, 1985; Roninson *et al.*, 1986; Fuqua *et al.*, 1987) or cells transfected with the gene encoding for Pgp (Gros *et al.*, 1986; Sugimoto and Tsuruo, 1987; Ueda *et al.*, 1987), there are now several reports on the expression of Pgp *in vivo*, in fresh tumor cells or in normal and neoplastic tissues from animals or cancer patients (Thorgeirsson *et al.*, 1987; Croop *et al.*, 1989; Thiebaut *et al.*, 1987; Fojo *et al.*, 1987; Pastan and Gottesman, 1987; Sugawara *et al.*, 1988). Pgp expression, at least for some human tumors, appears to play an important role in resistance to anticancer agents, and thus ultimately the failure of therapy. It would therefore be useful to develop therapeutic strategies to avoid MDR, and if possible to restore the sensitivity of resistant cancer cells. Various compounds differing in chemical structure and pharmacological properties, including calcium antagonists, cyclosporins, hormones, antiarrhythmic drugs and antidepressants have been found to be efficient inhibitors of Pgp, totally or partially reversing the drug resistance of cancer cell lines (for a review see Ford and Hait, 1990). One problem for their clinical use is that these revertant agents are often effective *in vitro* at concentrations which are too high to be safely achieved *in vivo*. For example, verapamil, which is certainly the revertant agent most widely studied *in vitro*, reverses the MDR of several cancer cell lines at concentrations which are cardiotoxic *in vivo* (Ozols *et al.*, 1987).

However, the mechanism of toxicity and the mechanism by which the revertant agents counteract MDR are not necessarily the same, thus suggesting that nontoxic revertant agents can be theoretically developed. For example *in vitro* effective revertant concentrations of CsA can be achieved *in vivo* at doses with a low probability of toxicity, if given for a short time.

One further problem which could be encountered in attempting to restore the sensitivity of a resistant tumor by inhibiting Pgp *in vivo* is that the toxicity of the antitumor agent may be increased by concomitant treatment with the resistance modifier. This is suggested by the observation that Pgp expression is not confined to resistant tumor cells, but may arise in normal tissues in several species including humans (Croop *et al.*, 1989;

Received for publication November 11, 1993.
[1] This work was supported by a generous contribution of the Fondazione Angelo and Angela Valenti and by A.I.R.C., Milan, Italy. Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with national and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

ABBREVIATIONS: MDR, multidrug resistance; Pgp, P-glycoprotein; CsA, cyclosporin A; Dx, doxorubicin; AUC, area under the curve.

Fojo *et al.*, 1987; Pastan and Gottesman, 1987). Pgp is in fact expressed in relatively large amounts in hepatic biliary ducts, renal tubules, intestine, adrenal cortex and brain capillaries. In these tissues Pgp probably exerts an important protective function by pumping potential toxic xenobiotica out of the cells, thus possibly enhancing the rate of their elimination from the body. Possibly, therefore, a compound which effectively reverses MDR by inhibiting Pgp and increasing intracellular drug retention in cancer cells may well also increase the retention and toxicity of the same drug in some normal tissues.

There is virtually no information on *in vivo* interactions between revertant agents and antitumor drugs, in spite of the fact that these combinations are currently under clinical investigation in many cancer centers. This lack of information prompted us to undergo this study, designed to assess whether CsA, which is one of the most widely used revertant agents (Slater *et al.*, 1986; Ford and Hait, 1990; Yahanda *et al.*, 1992; Twentyman, 1992), modifies the distribution of Dx in normal tissues of mice and rats and, if so, to what extent.

The pharmacokinetic changes observed in both animal species suggest caution in applying these combinations in clinical practice without appropriate pharmacological and toxicity monitoring.

Materials and Methods

Animals and drugs. Crl/CD BR male rats (200 ± 20 g b.wt.) and CD_2F_1/Crl BR male mice (20 ± 2 g b.wt.) obtained from Charles River Italia (Calco, Italy) were used for these experiments.

CsA (Sandoz Ltd, Basel, Switzerland) was freshly dissolved in olive oil. Dx (Farmitalia-Carlo Erba, Milan, Italy) was freshly dissolved in distilled water.

Pharmacokinetic studies. CsA was injected i.p. to rats at the dose of 30 mg/kg. Dx was injected i.v. at the dose of 5 mg/kg (corresponding to 30 mg/m^2) in the tail vein 30 min after CsA. Five, 15, 30 and 60 min and 2, 6, 24 and 48 hr after Dx, four animals per point were killed by exsanguination under light anesthesia, and serum and tissues (brain, heart, liver, kidneys and adrenals) were removed and frozen at −20°C until use.

CsA was injected i.p. to mice at two different doses, 12.5 and 25 mg/kg. Dx was injected i.v. at the dose of 10 mg/kg (corresponding to 30 mg/m^2) 30 min after CsA. Five, 15, 30 and 60 min and 2, 6, 24, 48 and 72 h after Dx injection, six animals per point were killed by exsanguination under light ether anesthesia and serum and tissues (brain, heart, liver, small intestine, kidneys and adrenals) were removed and frozen at −20°C until use.

Three groups of mice (15 each) were housed in metabolic cages until 72 hr after Dx and CsA. Urine and feces were collected at different intervals (0–20, 20–48 and 48–72 hr) and immediately stored at −20°C until analysis. These mice were also used for assessing the toxicity of the treatment and the survival time.

Analytical assay. Dx and metabolites were quantified by high-performance liquid chromatography with fluorimetric detection according to a described technique (Broggini *et al.*, 1984) with minor modifications. Serum samples, with daunorubicin added as internal standard, were extracted with 8 ml of chloroform/isopropanol (1:1) and centrifuged at 3000 rpm; the organic phase was evaporated to dryness under vacuum. After homogenization in water, tissue samples, with daunorubicin added as internal standard, were deproteinized with $AgNO_3$ (33%), extracted with 8 ml of isopropanol and then processed as described for serum samples. Extracts were injected into the high-performance liquid chromatography with fluorescence detection at an excitation wavelength of 475 nm and an emission of 580 nm. Separation was done with an isocratic solvent system of water/acetonitrile/0.1 M phosphoric acid using a 30-cm μBondapak C18 (10-μm) column. Recovery of Dx and its metabolites was 85 ± 5% for serum and tissues and the sensitivity was 5 ng/ml of serum and 20 ng/g for tissues.

Pharmacokinetic and statistical analysis. The AUC of drug concentrations (μg/ml or g) as a function of time (hr) was calculated by the trapezoidal method. Statistical significance was assessed by Duncan's test.

Results

Dx distribution in the rat. The pharmacokinetic profiles of Dx in serum and tissues of rats given the drug alone or 30 min after CsA are illustrated in figure 1. Dx serum profiles were similar in rats pretreated or not with CsA. Dx serum levels declined following a triphasic decay with $t_{1/2}$, α, β and γ of 2 min, 15 min and 20 hr in the group treated with Dx alone, and 2 min, 19 min and 30 hr in the group given CsA and Dx.

These parameters can only be taken as indicative, as they were established from too few points for a precise calculation of the kinetic parameters of a triexponential equation. For example the longer terminal half-life of Dx after CsA treatment should be taken with caution as it is based only on the slightly higher Dx concentration at 48 hr. It was beyond not our aim to calculate the precise serum kinetic parameters, which would have required a much larger number of time points; our main focus being to investigate whether CsA treatment modified Dx tissue distribution.

There were marked differences in Dx tissue distribution in rats pretreated with CsA, which caused increases in Dx concentrations in liver, adrenals and kidney. The difference was clear-cut 24 and 48 hr after Dx treatment. Dx levels in brain were much lower than in other tissues, with no difference between CsA-treated or untreated rats. The levels at 48 hr and AUC values are set out in table 1. In liver, kidney, adrenals and heart

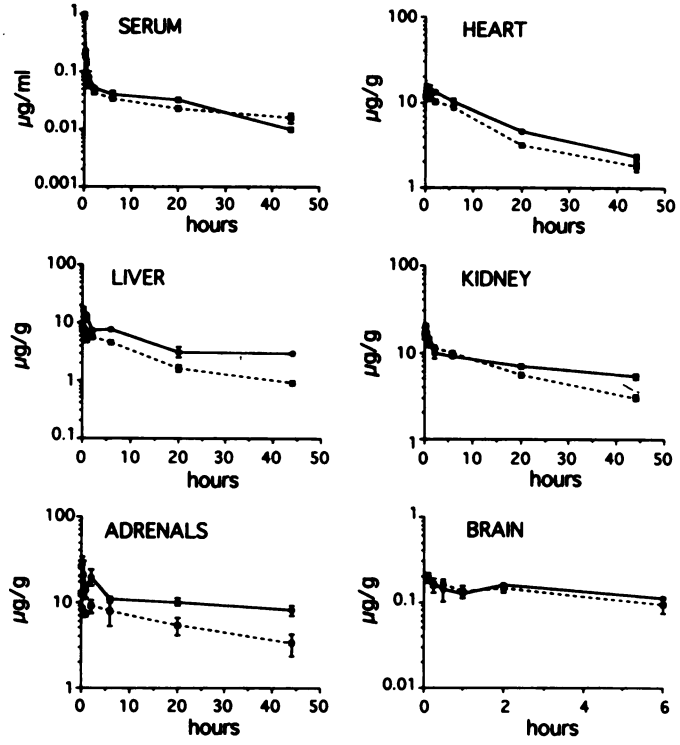

Fig. 1. Disappearance curves of Dx in serum and tissues of rats treated with Dx (5 mg/kg i.v.) alone ■ - - - - ■ or with CsA (30 mg/kg i.p.) ●——●. Bars represent S.E.; not visible when smaller than the symbols. For further details see table 1.

TABLE 1

Dx 48-hr levels and AUC in rats receiving the drug alone or in combination with CsA

Values are mean ± S.E. expressed in micrograms per milliliter for serum and micrograms per gram for tissues. AUC were calculated by trapezoidal integration up to 20 hr for serum and brain and up to 48 hr for the other tissues; they are expressed in micrograms per milliliter × hour for serum and micrograms per gram × hour for tissues. CsA was given i.p. at the dose of 30 mg/kg, 30 min before Dx. Dx was given i.v. at the dose of 5 mg/kg. Four animals per group were used for these studies.

	Dx		CsA + Dx	
	48-Hr Level	AUC	48-Hr Level	AUC
Serum	<0.02	0.78 ± 0.02	<0.02	0.97 ± 0.1
Heart	1.8 ± 0.2	201 ± 13	2.3 ± 0.2	260 ± 16**
Liver	0.9 ± 0.05	106 ± 8	2.9 ± 0.2**	202 ± 21**
Kidney	3.0 ± 0.2	278 ± 12	5.3 ± 0.4**	324 ± 17*
Adrenals	3.3 ± 0.9	243 ± 75	8.1 ± 1.1*	457 ± 55**
Brain	<0.02	0.76 ± 0.1	<0.02	0.82 ± 0.03

* $P < .05$ *vs.* controls, Duncan's test.
** $P < .01$ *vs.* controls, Duncan's test.

the Dx AUC were significantly higher in animals pretreated with CsA.

Dx distribution in the mouse. Further studies were carried out to see whether CsA pretreatment also markedly changed Dx distribution in tissues which express Pgp in mice. The pharmacokinetic profile in serum and some tissues of mice given the drug alone or 30 min after 12.5 or 25 mg/kg of CsA is shown in figure 2.

Dx serum levels appeared similar in the three groups and Dx was not detectable in serum 24 hr after drug treatment. In all tissues except brain Dx levels were higher 24, 48 and 72 hr after treatment with CsA (when Dx was not detectable in serum, *i.e.*, <5 ng/ml). The concentrations of Dx at 72 hr and Dx AUC values determined in serum and in tissues of mice given Dx alone or in combination with CsA at either 12.5 or 25 mg/kg are shown in table 2. In liver, small intestine, kidney and adrenals, Dx levels at 72 hr and AUC values were higher ($P < .01$) in mice pretreated with CsA. In the heart no significant difference was demonstrated at 72 hr but the AUC values were greater [$P < .05$ for CsA (12.5 mg/kg) and $P < .01$ for 25 mg/kg]. No significant differences were found between 72-hr levels and AUC in serum and brain of the three experimental groups. Although Dx levels and Dx AUC appeared higher in mice given 25 mg/kg of CsA than in those given 12.5 mg/kg, the differences were not in fact significant.

Dx metabolism. The levels of doxorubicinol and Dx-aglycone in livers of mice given Dx alone or after CsA are summarized in table 3. The concentration of both metabolites were higher in the liver of mice pretreated with CsA. The total difference is well illustrated by the AUC.

Excretion of Dx and metabolites. Renal and fecal elimination of Dx and metabolites accounted for less than 10 and 1% of the dose, respectively (table 4). The urinary elimination of Dx was reduced during the first 20 hr in animals treated with the higher dose of CsA; nevertheless the total amount of Dx excreted was similar for the three groups. Although doxorubicinol urinary elimination was higher in mice pretreated with CsA, the amounts excreted were almost negligible in terms of the Dx dose. Dx-aglycone was only present in traces, with no differences between the three groups.

Toxicity. CsA clearly increased the toxicity of Dx (fig. 3). All mice given Dx (10 mg/kg) alone and 90% of the animals receiving CsA (25 mg/kg) alone were alive 90 days after treatment, whereas 100 and 85% of mice died when Dx was given in combination with CsA, respectively, at the doses of 25 and 12.5 mg/kg.

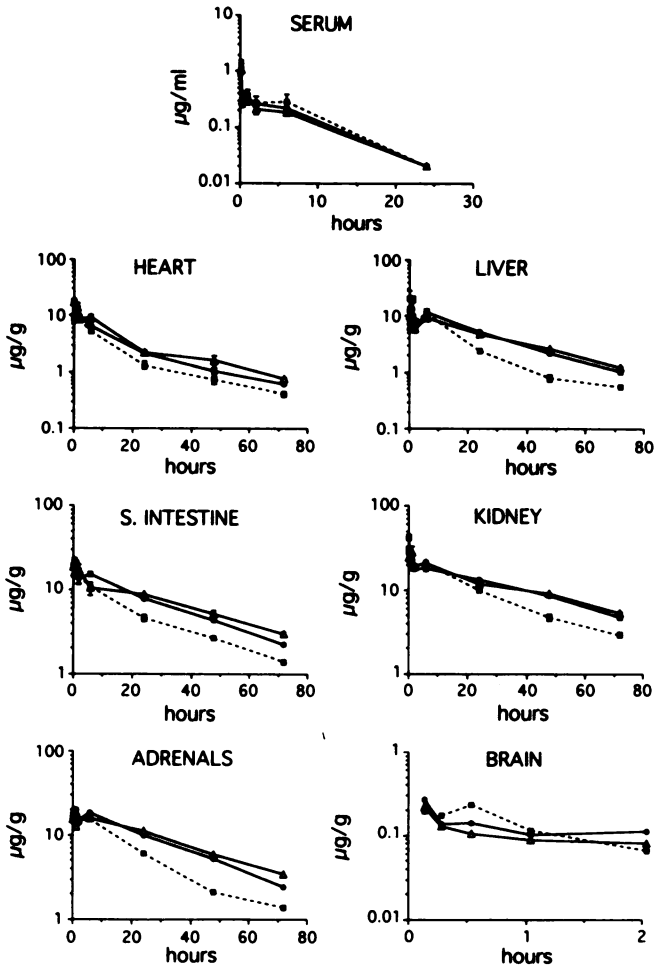

Fig. 2. Disappearance curves of Dx in serum and tissues of mice treated with DX (10 mg/kg i.v.) alone ■ - - - - ■ or with CsA at the i.p. doses of 12.5 ●——● and 25 Δ——Δ mg/kg). Bars represented S.E.; not visible when smaller than the symbols. For further details see table 2.

Discussion

The present study shows that CsA dramatically alters the distribution of Dx in rats and mice. Dx concentrations were increased most by CsA pretreatment in tissues known to express Pgp (Fojo *et al.*, 1987; Pastan and Gottesman, 1987; Cornwell, 1991) such as liver, adrenals, kidney or small intestine. Therefore the changes induced by CsA could be due to its ability to block Pgp, with a consequent decrease in the drug efflux from the tissues expressing this protein. In many cancer cell lines expressing Pgp, CsA can increase drug retention by a mechanism involving the inhibition of Pgp function, and this forms the basis for its use as an MDR revertant agent (Hait *et al.*, 1989; Foxwell *et al.*, 1989). The main changes in Dx concentrations were observed several hours after drug administration, suggesting that the mechanism involves a decrease in the rate of efflux from tissues rather than an increase in uptake and this is also in line with the majority of observations, using *in vitro* cultured cancer cells.

In the heart the concentrations of Dx were also higher in rats or mice treated with CsA although less so than in other

TABLE 2

Dx 72-hr levels and AUC in mice given Dx alone or in combination with CsA

Values are mean ± S.E. expressed in micrograms per milliliter for serum and micrograms per gram for tissues. AUC were calculated by trapezoidal integration up to 6 hr for serum and brain and up to 72 hr for tissues; they are expressed in micrograms per milliliter × hour for serum and micrograms per gram × hour for tissues. CsA was given i.p. at the doses of 12.5 or 25 mg/kg, 30 min before Dx. Dx was given i.v. at the dose of 10 mg/kg. Six animals per group were used for these studies.

	Dx		CsA 12.5 + Dx		CsA 25 + Dx	
	72-Hr Level	AUC	72-Hr Level	AUC	72-Hr Level	AUC
Serum	<0.02	1.8 ± 0.4	<0.02	1.7 ± 0.4	<0.02	1.4 ± 0.2
Heart	0.4 ± 0.01	144 ± 11	0.62 ± 0.03	188 ± 15*	0.76 ± 0.02	233 ± 29**
Liver	0.55 ± 0.03	239 ± 13	1.01 ± 0.05**	332 ± 22**	1.25 ± 0.04**	307 ± 14*
Intestine	1.4 ± 0.01	356 ± 26	2.20 ± 0.07**	522 ± 21**	3 ± 0.07**	522 ± 42**
Kidney	2.9 ± 0.2	657 ± 43	4.7 ± 0.3**	816 ± 42**	5.3 ± 0.2**	844 ± 29**
Adrenals	1.4 ± 0.4	420 ± 32	2.4 ± 0.1**	623 ± 22**	3.4 ± 0.8**	648 ± 48**
Brain	<0.02	0.23 ± 0.06	<0.02	0.21 ± 0.04	<0.02	0.17 ± 0.03

* $P < .05$ *vs.* controls, Duncan's test.
** $P < .01$ *vs.* controls, Duncan's test.

TABLE 3

Levels of doxorubicinol and Dx aglycone in liver of mice receiving Dx alone or in combination with CsA

Values are mean ± S.E. expressed in micrograms per gram. Dx and CsA were given as described in table 2. ND, not detectable. AUC were calculated by trapezoidal integration up to 6 hr for doxorubicinol and up to 72 hr for Dx aglycone; they are expressed in micrograms per gram × hour. Six animals per group were used.

Time	Doxorubicinol			Dx Aglycone		
	Dx	CsA 12.5 + Dx	CsA 25 + Dx	Dx	CsA 12.5 + Dx	CsA 25 + Dx
5 min	1.1 ± 0.1	1.9 ± 0.2*	2.1 ± 0.3**	13.2 ± 0.5	19.6 ± 1.7*	25 ± 2.7**
15 min	2.0 ± 0.3	2.8 ± 0.4	3.1 ± 0.2*	23.4 ± 1.4	31.4 ± 1.8*	36.6 ± 1.8**
30 min	1.9 ± 0.1	2.4 ± 0.4	1.9 ± 0.1	30.7 ± 0.3	28.9 ± 0.4	23.7 ± 2.7
1 hr	1.5 ± 0.8	2.4 ± 0.4*	2.3 ± 0.1*	17.6 ± 1.0	25.0 ± 3.6	26.8 ± 0.7*
2 hr	1.4 ± 0.2	1.8 ± 0.1	1.9 ± 0.2	22.4 ± 0.7	23.7 ± 0.2	28.4 ± 2.5*
6 hr	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	5.8 ± 0.1	6.9 ± 0.4	7.9 ± 0.5**
24 hr	N.D.	N.D.	N.D.	2.9 ± 0.5	4.3 ± 0.5	3.6 ± 0.2
48 hr	N.D.	N.D.	N.D.	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.2
72 hr	N.D.	N.D.	N.D.	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
AUC	7.0 ± 1	9.4 ± 1**	9.7 ± 1**	241 ± 18	293 ± 24**	305 ± 21**

* $P < .05$ *vs.* controls, Duncan's test.
** $P < .01$ *vs.* controls, Duncan's test.

TABLE 4

Urinary and fecal excretion of Dx and doxorubicinol (Dxol) in mice receiving Dx alone or in combination with CsA

The values are mean ± S.E. of three groups each of five mice. Dx and CsA were given as described in table 2. For further details see under "Materials and Methods." N.D., not detectable.

	Urine									
	0 to 20 Hr		20 to 48 Hr		48 to 72 Hr		Cumulative		Percentage of the Dose	
	Dx	Dxol	Dx	Dxol	Dx	Dxol	Dx	Dxol	Dx	Dxol
Dx	59 ± 7	2.6 ± 0.3	16 ± 0.9	1.9 ± 0.1	6 ± 2	0.9 ± 0.2	81	5.4	8.1	0.5
CsA 12.5 + Dx	46 ± 7	2.1 ± 0.3	26 ± 1*	2.3 ± 0.1	12 ± 1	1.5 ± 0.1*	84	5.9	8.4	0.6
CsA 25 + Dx	36 ± 4*	2.1 ± 0.2	27 ± 3*	3.2 ± 0.4*	9 ± 3	1.6 ± 0.2*	72	6.9	7.2	0.7

	Feces				
	0 to 20 Hr	20 to 48 Hr	48 to 72 Hr	Cumulative	Percentage of the Dose
	Dx	Dx	Dx	Dx	Dx
Dx	4.0 ± 1.2	4.3 ± 0.3	N.D.	8.3	0.8
CsA 12.5 + Dx	4.6 ± 0.7	5.0 ± 0.1	0.5 ± 0.01	10.1	1.0
CsA 25 + Dx	4.0 ± 0.5	3.1 ± 0.6	0.5 ± 0.05	7.2	0.7

* $P < .05$ *vs.* controls, Duncan't test.

tissues. This finding is in line with the reported observation that Pgp expression in cardiac tissue is low (Fojo *et al.*, 1987).

CsA did not appear to modify Dx brain levels. This is an interesting finding in view of the report that Pgp might be involved in the blood-brain barrier (Cordon-Cardo *et al.*, 1989). In our conditions CsA did not modify the permeability of the brain to Dx. However, chronic administration of CsA might possibly affect brain Dx levels, even resulting in neurotoxicity as recently reported (Barbui *et al.*, 1992).

The effects of CsA on the tissue concentrations of Dx can be explained on the basis of differences in how long the drug persist in tissue, and do not necessarily involve other processes such as inhibition of metabolism or elimination. In fact in the liver at least, where the levels of doxorubicinol and Dx-aglycone could be quantitated, CsA pretreatment increased the concentrations of both metabolites, probably in relation to the increase in Dx concentration. Renal and fecal excretion of Dx or metabolites was not significantly reduced by CsA. Another possible mechanism of interaction could be the increase in free Dx fraction due to a displacement of plasma protein bound Dx by

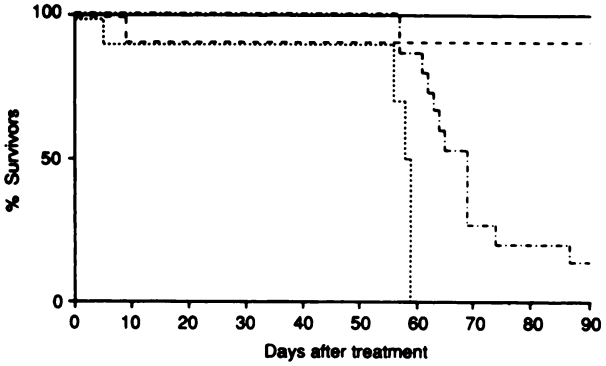

Fig. 3. Survival of mice treated with Dx alone (10 mg/kg i.v.) —— or CsA alone (25 mg/kg i.p.) - - - - - or with the combination of CsA (12.5 -·-·-· and 25 ..... mg/kg i.p.) and Dx (10 mg/kg i.v.).

CsA. This possibility is unlikely because the two drugs do not bind to the same plasma proteins. In fact Dx binds to albumin (to an extent of 74%) (Maniez-Devos *et al.*, 1985), whereas CsA binds to lipoproteins (at a rate of 21%) and only to a small percentage to other plasma proteins (Fahr, 1993).

The increase in Dx toxicity in mice pretreated with CsA was evident and is likely to be related to the changes in Dx concentrations in some tissues. The mechanism of the increased toxicity of Dx when combined with CsA has not been investigated in detail. However, preliminary data indicate that hepatotoxicity, assessed by evaluating serum transaminases, did arise in mice treated with the combination, but not with Dx alone. We have not determined CsA levels and therefore we cannot exclude that the increased toxicity is at least in part consequent to a change in CsA pharmacokinetics due to subsequent treatment with Dx. However a similar delayed toxicity was recently found giving a higher Dx dose (*i.e.*, 14 mg/kg i.v.) alone (data not shown), thus suggesting that the increased tissue levels of Dx are responsible for increased Dx toxicity when given with CsA. The toxicity data suggest caution in combining CsA and Dx in clinical use without a careful phase I clinical trial designed to establish the maximum tolerated doses of the two drugs.

Recently Lum *et al.* (1992) reported that CsA can cause marked changes in etoposide pharmacokinetics with much higher plasma AUC and a longer elimination half-life. The same authors demonstrated a concomitant increase in etoposide hematological toxicity in patients receiving high doses of CsA (Yahanda *et al.*, 1992). They suggested that CsA inhibited Pgp and reduced the outward transport of etoposide from normal tissues. In addition they speculated on the possibility that CsA might reduce etoposide metabolism and elimination. No data were obtained on etoposide tissue levels on account of the ethical problems of obtaining tissue biopsies from patients for pharmacological studies.

The results of the present study, in rats and mice, confirm that CsA can profoundly change the pharmacokinetics of drugs known to be substrates for Pgp transport protein.

Serum concentrations of Dx are known to be much lower than those reached in normal and neoplastic tissues of mice, rats or humans (Broggini *et al.*, 1980; Colombo *et al.*, 1989; Rossi *et al.*, 1987). This explains why in mice and rats no or only small changes in Dx serum levels of animals could be detected after CsA in spite of the dramatic changes in some tissues. This might have been partially due to the fact that Dx was no longer detectable in serum 24 hr after treatment, when the differences in Dx tissues levels became most marked. Nevertheless these results do indicate that differences in tissue distribution of an anticancer agent, induced by CsA or any other "revertant agent," can be much more marked than might be expected on the basis of serum drug levels alone. This should be kept in mind in clinical investigations with the combination of revertant agents and anticancer drugs to avoid underestimating the risk of toxicity, based on the limited changes in drug plasma levels.

Studies are now in progress in mice bearing tumors expressing Pgp to see whether the increase in Dx toxicity, when given after CsA, is counterbalanced by a much greater antitumor activity. Studies are also in progress to evaluate whether the interaction is unique for CsA, or also applies to its nonimmunosuppressor analog SDZ PSC 833 (Friche *et al.*, 1992; Keller *et al.*, 1992; Jonsson *et al.*, 1992), or to other structurally unrelated agents known to counteract MDR by inhibiting Pgp function.

Acknowledgments

The kind help of Dr. Donatella Gentili and Dr. Luigi Imperatori is gratefully acknowledged.

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Send reprint requests to: Maurizio D'Incalci, M.D., Laboratory of Cancer Chemotherapy, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.