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ORIGINAL ARTICLE

**Jaime Cárcel-Trullols · Francisca Torres-Molina
Amparo Araico · Anas Saadeddin · José Esteban Peris**

Effect of cyclosporine A on the tissue distribution and pharmacokinetics of etoposide

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Abstract *Purpose*: Cyclosporine A (CyA) is able to inhibit P-glycoprotein (P-gp) and to increase cytotoxicity of some anticancer drugs, including etoposide. However, the effect of CyA on the distribution of etoposide in normal tissues, which could affect their toxicity, has not been studied extensively. The purpose of this study was to investigate the effect of CyA on the pharmacokinetics and tissue distribution of etoposide in rats. *Methods*: Etoposide was administered as an i.v. bolus injection (3 mg) or as a constant-rate i.v. infusion (8 mg/h) 1 h after the beginning of infusion of CyA or saline. Animals were killed 1 h after the bolus administration or after the beginning of infusion of etoposide, and plasma and tissue (testicle, muscle, heart, lung, spleen, kidney, liver, colon, and intestine) concentrations of etoposide, blood concentrations of CyA were determined. All analyses were performed by HPLC. *Results*: Infusion of CyA (1 mg/h) in rats treated with an i.v. bolus of etoposide caused a decrease in the plasma clearance (5.4 ± 2.1 vs 9.3 ± 2.4 ml/min), and an increase in plasma and tissue concentrations of etoposide, but the tissue-to-plasma concentration ratios of etoposide were not affected. When etoposide was infused at a constant rate to reach a steady-state plasma level, coinfusion of CyA (10 mg/h) also caused a decrease in the plasma clearance (4.8 ± 1.5 vs 9.8 ± 4.7 ml/min), and an increase in plasma and tissue concentrations of etoposide. Only lung and spleen showed tissue-to-plasma ratios of etoposide significantly higher than obtained in rats coinfused with saline, but the differences were small. *Conclusions*: The higher tissue concentrations of etoposide caused by CyA administration were mainly a direct consequence of the higher plasma concentration resulting from a decrease in the clearance of etoposide rather than a consequence of changes in the tissue distribution of etoposide. Extrapolation of the results obtained in rats to clinical practice suggests that the coadministration of etoposide and CyA would not lead to an increase in the toxicity of etoposide if the dose were decreased in the same proportion as clearance of etoposide is decreased by CyA administration.

Keywords P-glycoprotein · Cyclosporine A · Etoposide · Tissue distribution · Pharmacokinetics

J. Cárcel-Trullols · F. Torres-Molina · A. Araico
A. Saadeddin · J. E. Peris (✉)
Department of Pharmacy and Pharmaceutical Technology,
Faculty of Pharmacy, University of Valencia,
Avda. V.Andrés Estellés s/n, Burjassot, 46100, Valencia, Spain
E-mail: jose.e.peris@uv.es
Tel.: +34-96-3544914
Fax: +34-96-3544911

Introduction

Etoposide is a semisynthetic podophyllotoxin that is used as a component of various chemotherapeutic regimens for the treatment of refractory testicular tumors, lung cancer, malignant lymphomas and Hodgkin's disease, leukemia, Wilm's tumor, neuroblastoma, and AIDS-related Kaposi's sarcoma. As with other anticancer agents, etoposide toxicity includes hematological toxicity (myelosuppression), gastrointestinal effects (nausea and vomiting), cardiovascular effects, and hepatotoxicity [1].

A major problem in the treatment of patients with cancer is the occurrence of resistance to anticancer drugs. In the case of etoposide, one of the major acquired drug resistance mechanisms involves the classical multidrug resistance (MDR) accompanied by the synthesis of P-glycoprotein (P-gp), a member of the superfamily of ATP-binding cassette (ABC) transporters [2–4]. Although P-gp was initially isolated due to its role in MDR to cancer chemotherapeutics, later work showed that this transporter is also involved in the pharmacokinetics of many drugs. P-gp is expressed in the luminal epithelial cells of organs often associated with drug absorption and disposition, such as the

hepatocyte canalicular membrane, renal proximal tubules, the small and large intestine, brain, testis, and adrenal glands [5–7]. Its physiological function is thought to be an excretory or protective one.

Cyclosporine A (CyA) has been shown to reverse, at least in part, P-gp-mediated drug efflux [8], and has recently undergone preclinical and clinical testing as a modulator of MDR [9–14].

In vitro studies with drug-resistant HeLa cells, in which P-gp was induced, have shown that resistance to several anticancer drugs, including etoposide, is abolished by CyA [14]. Since CyA is able to reverse P-gp-mediated drug efflux, this abolished resistance could be a consequence of higher intracellular concentrations of etoposide in the presence of CyA. Indeed, in vitro studies with leukemic cells have shown that intracellular retention of radioactivity from ^{3}H-etoposide is increased by a factor of 1.5 at the most in the presence of CyA [15].

It has also been reported that CyA produces a concentration-dependent decrease in renal and nonrenal elimination of etoposide and increases etoposide-induced myelosuppression [10, 11, 16–18]. Although an effect of CyA on the elimination of etoposide has been reported, the effect of CyA on the distribution of etoposide in normal tissues has not been studied comprehensively. While enhancement of tissue penetration of anticancer drugs could be beneficial in the treatment of primary and metastatic tumors, it could also enhance the organ-related toxicity of these drugs.

The aim of the present study was to investigate the effect of CyA on the clearance and tissue distribution of etoposide using a rat model. CyA was intravenously administered as a constant-rate infusion in order to obtain steady-state blood levels of the drug. Etoposide was administered as an intravenous bolus injection and also as a constant-rate intravenous infusion.

Materials and methods

Chemicals and animals

Etoposide (Vepesid) and CyA (Sandimmune) were purchased from Bristol-Myers Squibb (Madrid, Spain) and Novartis (Barcelona, Spain), respectively. Both drugs were diluted with saline prior to administration to the animals. All the other chemicals used were of analytical or HPLC grade.

Adult male Wistar rats, weighing between 300 and 330 g, were used in this study and the experiments were performed in accordance with the rules of our university for the use of animals for research purposes. Animals were maintained under a 12 h/12 h light/dark cycle and had free access to food and water before the experiments. The day before drug administration, the animals were subjected to the cannulation of both jugular veins in order to facilitate intravenous drug administration and blood sampling [19].

Experiments

To evaluate the effect of CyA on the pharmacokinetics of etoposide, steady-state blood levels of CyA were obtained by means of a constant-rate infusion of CyA, and thereafter, etoposide was intravenously administered by either bolus injection or constant-rate infusion (Table 1).

The control groups consisted of rats intravenously infused with saline instead of CyA and treated with etoposide (bolus or constant-rate infusion). The plasma and tissue concentrations of etoposide obtained in rats infused with CyA were compared with the concentrations obtained in the rats belonging to the corresponding control group. All groups were composed of six rats.

Intravenous bolus injection of etoposide

A 3-mg dose of etoposide was administered to rats infused with saline (group 1) or CyA (group 2). Animals of group 1 (control) were infused with saline (1 ml/h) through the cannula implanted in the left jugular vein, and etoposide was administered through the right jugular vein (3 mg dissolved in 0.5 ml saline) 60 min after the start of the saline infusion. Animals of group 2 were given a loading dose of 0.5 mg CyA in 0.5 ml saline through the cannula implanted in the left jugular vein, and immediately thereafter, a constant-rate infusion of CyA (1 mg/h from a solution of 1 mg/ml) was started through the same cannula. After 60 min of CyA infusion, etoposide was administered as indicated for group 1.

Blood samples (0.3–0.4 ml) were collected with heparinized syringes through the cannula implanted in the right jugular vein at 5, 10, 20, 40 and 60 min after the administration of etoposide. Aliquots (0.1 ml) of whole blood taken after 20, 40 and 60 min were separated from the samples before centrifugation and were used to determine the concentration of CyA. Blood samples were centrifuged (2000 *g* for 5 min) and the plasma was used to determine the concentration of etoposide.

After the last blood sampling, rats were killed by injection of a saturated solution of KCl (0.5 ml) and samples of the following tissues were obtained: testicle, muscle, heart, lung, spleen, kidney, liver, colon, and intestine. The concentration of etoposide was determined in all these tissues.

Prior to these experiments, three rats were treated intravenously with 0.5 mg CyA followed by a constant-rate infusion of 1 mg/h of CyA. Blood samples

Table 1 Experimental groups ($n = 6$ in each group)

Group	Etoposide administration	CyA administration
1 (control)	Bolus (3 mg)	–
2	Bolus (3 mg)	Infusion (1 mg/h)
3 (control)	Infusion (8 mg/h)	–
4	Infusion (8 mg/h)	Infusion (1 mg/h)
5	Infusion (8 mg/h)	Infusion (10 mg/h)

withdrawn at 60, 90 and 120 min showed an apparent steady-state blood concentration of CyA (3.0 ± 0.7, 2.9 ± 0.8, and 3.2 ± 1.3 μg/ml) reached at the time of etoposide administration (60 min after the start of CyA infusion), and maintained during the following 60 min.

Intravenous constant-rate infusion of etoposide

Rats in group 3 (control) were infused with saline as indicated for group 1. After 60 min, a loading dose of 3 mg etoposide was administered through the left jugular vein cannula and a constant-rate infusion (8 mg/h from an 8 mg/ml solution) of etoposide was started. During the next 60 min, both infusions (saline and etoposide) were maintained through the left jugular vein cannula. Blood samples were withdrawn through the right jugular vein cannula at 30, 45 and 60 min after the start of etoposide infusion. The concentration of etoposide was determined in the plasma of these three samples. After the last blood sampling, rats were killed, tissue samples were taken as indicated above and analyzed for etoposide concentration.

The loading dose of etoposide was selected as the intravenously administered dose required to yield an immediate plasma etoposide concentration similar to the steady-state plasma concentration reached during the constant-rate infusion. An estimation of the steady-state plasma concentration of etoposide was obtained from the ratio between the infusion rate (8 mg/h) and the total plasma clearance obtained in rats of group 1. The estimated steady-state plasma concentration (14 μg/ml) was similar to the initial plasma concentration obtained after the administration of a 3-mg dose (about 15 μg/ml; group 1), and this dose of etoposide was therefore selected as the loading dose.

Rats in group 4 were infused with CyA (1 mg/h) as indicated for rats in group 2. After 60 min, a loading dose was administered followed by a constant-rate infusion (8 mg/h) of etoposide as indicated for group 3. During the next 60 min CyA and etoposide were coinfused at the indicated rates. Blood and tissues samples were obtained as indicated for group 3. The concentration of CyA was determined in aliquots of the blood samples, and the concentration of etoposide was determined in the plasma of these samples and in the tissue samples.

Rats in group 5 were treated as indicated for group 4, with the only difference being that the infusion rate of CyA was 10 mg/h (from a 10 mg/ml solution) instead of 1 mg/h, and the loading dose was 5 mg instead of 0.5 mg. Blood and tissues samples were withdrawn as indicated for group 3.

Analytical methods

The equipment used was a Waters (Milford, Mass.) HPLC chromatograph composed of the following modules: a Waters 515 pump, a 7725i Rheodyne injector, a Waters Concorde electrochemical detector containing a VT-03 flow cell with an in situ Ag/AgCl reference electrode, a Waters 486 UV detector, and a Waters 743 data module. The analytical column was a Nova Pak C_{18} cartridge of 15 cm×3.9 mm i.d.

Etoposide

Plasma samples were extracted according to the following procedure. Plasma (0.1 ml) was added to 0.1 ml water and 1 ml extraction solution (chloroform/isopropanol, 95:5, v/v) and shaken for 15 min in a DSG-301 Heidolph shaker. After centrifugation (2000 *g* for 5 min), 0.9 ml of the organic phase was placed in a glass tube and evaporated to dryness in a water bath. The residue was dissolved in 100 μl mobile phase and an aliquot of 20 μl was injected into the HPLC system. The mobile phase consisted of acetate buffer (50 m *M*, pH 4), methanol and acetonitrile (60/35/5, v/v/v), containing EDTA (0.1 mg/ml) and KCl (0.15 mg/ml). The mobile phase was delivered at 1 ml/min and detection of etoposide was performed at a potential of $+0.7$ V and an oven temperature of 35°C. Calibration standards for etoposide (1, 5, 10, 20, and 50 μg/ml) were prepared in plasma and extracted as indicated above. The calibration curve was constructed by plotting the height of the peak corresponding to etoposide vs the nominal concentration, and a straight line was obtained. The concentration of etoposide in the plasma samples was obtained by interpolation on the calibration curve of the peak-height value corresponding to etoposide. The limit of detection (approximately 0.2 μg/ml) was determined as the etoposide concentration in plasma samples giving rise to a signal-to-noise ratio of 3. The limit of quantification (1 μg/ml) was set at the lowest standard concentration on the calibration curve. The coefficient of variation of the analytical method was less than 5%.

Tissue samples were blotted to remove superficial blood, weighed and homogenized with a 1.5-fold excess of saline in an Ultra-Turrax homogenizer. Aliquots (0.2 ml) of tissue homogenate were added to 1 ml extraction solution and processed as indicated for plasma samples to determine tissue etoposide concentrations.

Cyclosporine A

For the analysis of CyA, the analytical column was placed in a water bath at 70°C and the mobile phase consisted of a mixture of water, methanol, and acetonitrile (36/36/28, v/v/v). The mobile phase was delivered at 1 ml/min through the analytical column and detection of CyA in the effluent was performed with the UV detector set at 210 nm.

Blood samples containing CyA were subjected to the following extraction procedure. Water (0.1 ml) was added to 0.1 ml blood sample in an Eppendorf tube, the

mixture was shaken for 10 s by hand and 1 ml ethyl ether was added. After shaking the mixture for 30 min in a Heidolph shaker, it was centrifuged (2000 *g* for 5 min) and frozen at −16°C. After freezing of the lower aqueous phase, 0.8 ml of the upper organic phase was placed in a glass tube and dried in a water bath. The residue was redissolved in 0.1 ml of the mobile phase solution and an aliquot of 20 µl was injected into the chromatograph. Calibration standards for CyA (2, 5, 10, 25, and 50 µg/ml) were prepared in blood and subjected to the extraction procedure described above. The calibration curve was constructed by plotting the height of the peak corresponding to CyA vs the nominal concentration, and a straight line was obtained. The concentration of CyA in the blood samples was obtained by interpolation in the calibration curve of the peak-height value corresponding to CyA. The coefficient of variation of the analytical method was less than 5%, the limit of detection was approximately 0.6 µg/ml, and the limit of quantification was 2 µg/ml.

Pharmacokinetic methods and statistics

Intravenous bolus injection of etoposide

For each rat of groups 1 and 2, the following pharmacokinetic parameters of etoposide were calculated by noncompartmental methods [20]: terminal disposition half-life ($t_{1/2}$), steady-state volume of distribution (V_{ss}), mean residence time (MRT) and total plasma clearance (Cl). Previously, the total area under the plasma drug concentration–time curve (AUC) and the total area under the first moment curve (time×concentration vs time curve, AUMC) were calculated by means of a combination of the regular trapezoidal and the logarithmic trapezoidal rules [21], and the following equations were subsequently applied:

$$V_{ss} = \frac{\text{Dose} \times \text{AUMC}}{\text{AUC}^2}$$

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

$$\text{Cl} = \frac{\text{Dose}}{\text{AUC}}$$

The tissue-to-plasma concentration ratios (*R*) of etoposide were calculated by dividing the tissue concentration of etoposide and the plasma concentration determined immediately before killing the rat (60 min after etoposide dosing).

Statistical comparison of pharmacokinetic parameters and *R* values obtained in the two groups of animals (groups 1 and 2) was performed by means of Student's *t*-test, except in the case of parameters or *R* values with a nonhomogeneous variance in both groups (Snedecor *F*-test), which were compared by means of the Mann–Whitney *U*-test. In all statistical comparisons, a probability of less than 0.05 was considered to be statistically significant.

Intravenous constant-rate infusion of etoposide

Total plasma clearance of etoposide (Cl) in rats of groups 3, 4, and 5 was calculated as the ratio between the infusion rate of etoposide and the plasma concentration at steady-state (Cp_{ss}), measured 60 min after the beginning of the infusion. The tissue-to-plasma concentration ratios (*R*) of etoposide were calculated, as indicated above, using the plasma concentration at steady-state (Cp_{ss}).

Cl, Cp_{ss}, and *R* values obtained in the three groups were statistically compared by means of one-way ANOVA, followed by the multiple comparison Student-Newman-Keules test. Previously, homogeneity of variance in the three groups for the parameter to be compared was checked with the Bartlett test. In the case of a nonhomogeneous variance, the statistical comparison was performed by the Kruskal-Wallis test followed by the Mann-Whitney *U*-test. As indicated before, a probability of less than 0.05 was considered to be statistically significant.

Results and discussion

Intravenous bolus injection of etoposide

The plasma concentrations obtained after the intravenous bolus injection of etoposide (Fig. 1) were higher in the rats infused with CyA (group 2) than in the rats infused with saline (group 1), which indicates a reduced plasma clearance of etoposide in the rats infused with CyA.

The mean plasma clearance of etoposide estimated in group 1 was significantly higher than that estimated in group 2 (Table 2).

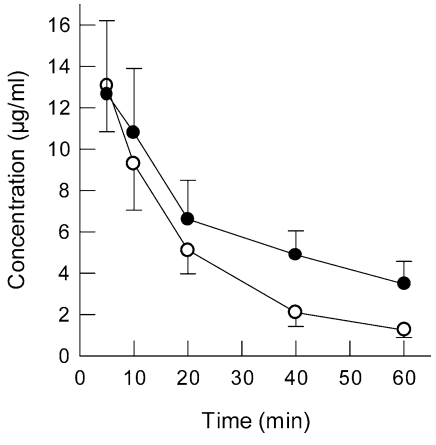

Fig. 1 Mean plasma levels and standard deviations of etoposide after intravenous bolus administration of 3 mg to rats infused with saline (*open circles* control group) or 1 mg/h of CyA (*closed circles* group 2)

Table 2 Pharmacokinetic parameters (mean ± SD, $n = 6$) of etoposide after intravenous bolus administration of a 3-mg dose to rats infused with saline (control) or CyA (1 mg/h)

Parameter	Control (group 1)	CyA (1 mg/h) (group 2)
Cl (ml/min)	9.3 ± 2.4	5.4 ± 2.1*
V_{ss} (ml)	218 ± 37	266 ± 81
$t_{1/2}$ (min)	20 ± 3	39 ± 4*
MRT (min)	24 ± 3	53 ± 10*
Cp (μg/ml)[a]	1.3 ± 0.4	3.5 ± 1.1*
Cb_{ss}-CyA (μg/ml)[b]	–	4.3 ± 1.8

*($P < 0.05$ vs corresponding control group value.
[a]Plasma concentration of etoposide 60 min after bolus administration.
[b]Steady-state blood concentration of CyA determined 60 min after bolus administration of etoposide

Regarding the other pharmacokinetic parameters, a statistically significant increase in $t_{1/2}$ and MRT of etoposide was observed in group 2. Although V_{ss} was slightly higher in group 2 than in group 1, the difference was not statistically significant. Blood concentrations of CyA at 20, 40, and 60 min after the intravenous bolus injection of etoposide (group 2) were 3.6 ± 1.2, 3.5 ± 1.5, and 4.3 ± 1.8 μg/ml, respectively. The changes in the pharmacokinetic parameters of etoposide caused by the infusion of CyA are similar to those observed by other authors in humans. Lum and Kaubisch [17] reported that CyA concentrations higher than 2.0 μg/ml produce a 38% decrease in total clearance of etoposide (−42% in this work), more than a twofold increase in $t_{1/2}$ (+95% in this work), and 46% increase in V_{ss} (+22% in this work) compared to etoposide alone. These findings suggest that the rat is a suitable animal model to study the pharmacokinetic interactions between CyA and etoposide, and that extrapolation of the results to humans is appropriate.

In most of the analyzed tissues, etoposide concentrations were higher in the rats of group 2 than in the rats of group 1 (Fig. 2).

These higher tissue concentrations of etoposide could be a direct consequence of the higher plasma concentration of etoposide observed in group 2 and/or the effect of CyA on the tissue distribution of etoposide. However, the tissue-to-plasma concentration ratios (R) of etoposide in the rats infused with CyA were similar to or lower than the corresponding values obtained in the control rats, which suggests that the higher etoposide concentrations in tissues of rats of group 2 were mainly due to the higher plasma levels of etoposide in these animals.

The concentration of etoposide in intestine was clearly higher than in the other tissues of both groups. Nevertheless, this tissue concentration in the intestine is not the "true" concentration in the tissue. Etoposide is excreted in bile [22, 23] and, although the intestine was opened and cleared of intestinal contents by mechanical removal of debris, residual bilis containing etoposide could have remained in the tissue. Therefore, the etoposide concentration determined in the intestine could have been affected by the concentration of etoposide in the excreted bile. This could explain why the apparent concentration in the intestine in rats infused with CyA was similar to or lower than the concentration in the intestine of control rats, in spite of the higher plasma concentration of etoposide. CyA inhibits biliary excretion of etoposide, resulting in a lower concentration in bile of rats infused with CyA than in bile of control rats.

Constant-rate intravenous infusion of etoposide

The etoposide tissue distribution following intravenous bolus injection of the drug could have been affected by the fact that the equilibrium in the distribution of the drug had not been reached at the time the rats were killed (60 min after etoposide dosing). For this reason,

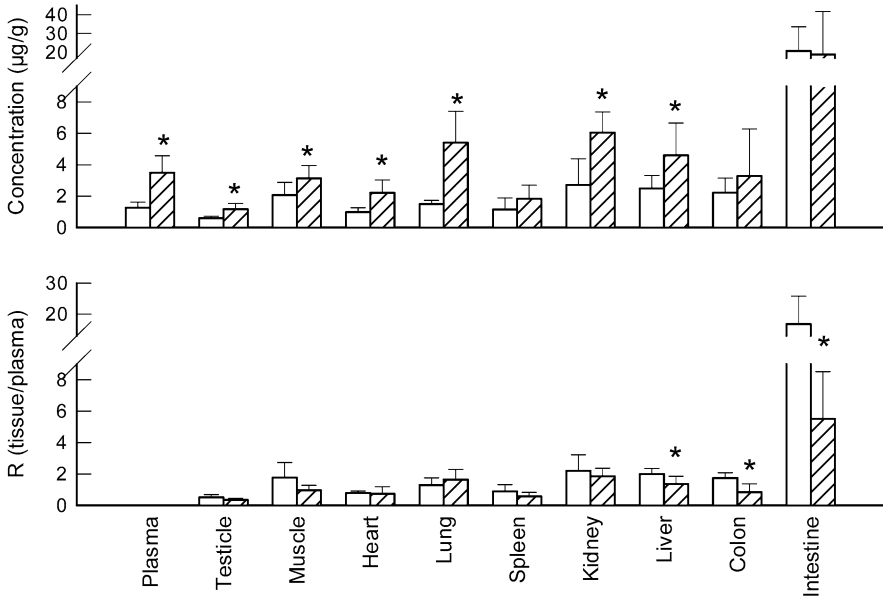

Fig. 2 *Top*: plasma and tissue concentrations (mean + SD) of etoposide determined 60 min after intravenous bolus administration (3 mg) to rats infused with saline (*open bars*, control group) or 1 mg/h of CyA (*hatched bars*, group 2). *Bottom*: tissue-to-plasma concentration ratios of etoposide. *$P < 0.05$ vs corresponding control group value

other groups of rats were treated with etoposide as an intravenous constant-rate infusion (groups 3, 4, and 5) in order to determine the clearance and tissue distribution of etoposide under steady-state conditions. Plasma concentrations of etoposide (16.8 ± 6.6, 18.1 ± 8.3, and 16.8 ± 7.8 μg/ml) and blood concentrations of CyA (2.9 ± 2.3, 3.6 ± 2.1, and 3.6 ± 2.0 μg/ml) determined at 30, 45, and 60 min after the start of etoposide infusion (group 4) showed that a steady-state had been reached for both drugs. As can be seen in Table 3 and Fig. 3, infusion of CyA at the constant-rate of 1 mg/h did not change the clearance or the tissue concentration of etoposide infused at the constant-rate of 8 mg/h in comparison to the group of animals infused with saline instead of CyA.

This result is clearly different from the one obtained in group 2, where rats were also infused with 1 mg/h of CyA. It should be pointed out that, at the time the rats were killed, the plasma concentration of etoposide after intravenous infusion was higher than the plasma concentration after intravenous bolus administration. Etoposide plasma concentrations in the control groups at the time the rats were killed were 15.9 ± 6.5 μg/ml in group 3 and 1.3 ± 0.4 μg/ml in group 1, whereas the CyA concentrations in groups infused at the constant-rate of 1 mg/h (groups 2 and 4) were approximately 4 μg/ml in both groups (4.3 ± 1.8 μg/ml in group 2, 3.6 ± 2.0 μg/ml in group 4). It follows that the ratio of CyA blood concentration to etoposide plasma concentration in the absence of CyA was approximately 0.3 (infusion, Table 3) and 3 (bolus, Table 2), and the lower ratio in the group of rats intravenously infused with etoposide could explain the absence of a detectable effect of CyA on the pharmacokinetics of etoposide in group 4.

An additional group of rats (group 5) were infused with CyA at a ten times higher rate (10 mg/h) in order to reach a steady-state blood concentration of CyA approximately three times higher than the etoposide concentration in the control group. The steady-state blood concentration of CyA in group 5 was approximately 20 times higher than that obtained in group 4, instead of the expected increase of ten times based on the ratio of infusion rates of CyA. This indicates nonlinear pharmacokinetics of CyA, with a decrease in clearance as dose (infusion rate) increases.

Some authors have reported linear pharmacokinetics of CyA [24] or a dose-dependent increase of total blood clearance [25] after intravenous bolus administration in rats. However, more extensive studies, in which CyA pharmacokinetics appeared to be essentially linear based on total blood concentration data, have shown multiple nonlinear factors in the pharmacokinetics of CyA [26,

Table 3 Plasma clearance of etoposide (Cl), steady-state plasma etoposide concentration (Cp_{ss}) and steady-state blood CyA concentration (Cb_{ss}-CyA) (mean ± SD, $n = 6$) after infusion of etoposide (8 mg/h) to three groups of rats coinfused with saline (control) or CyA (1 and 10 mg/h)

Parameter	Control (group 3)	CyA (1 mg/h) (group 4)	CyA (10 mg/h) (group 5)
Cl (ml/min)	9.8 ± 4.7	10.0 ± 5.5	4.8 ± 1.5*
Cp_{ss} (μg/ml)	15.9 ± 6.5	16.8 ± 7.8	28.1 ± 7.2*
Cb_{ss}-CyA (μg/ml)	–	3.6 ± 2.0	78.9 ± 11.8

*$P < 0.05$ vs corresponding control group value

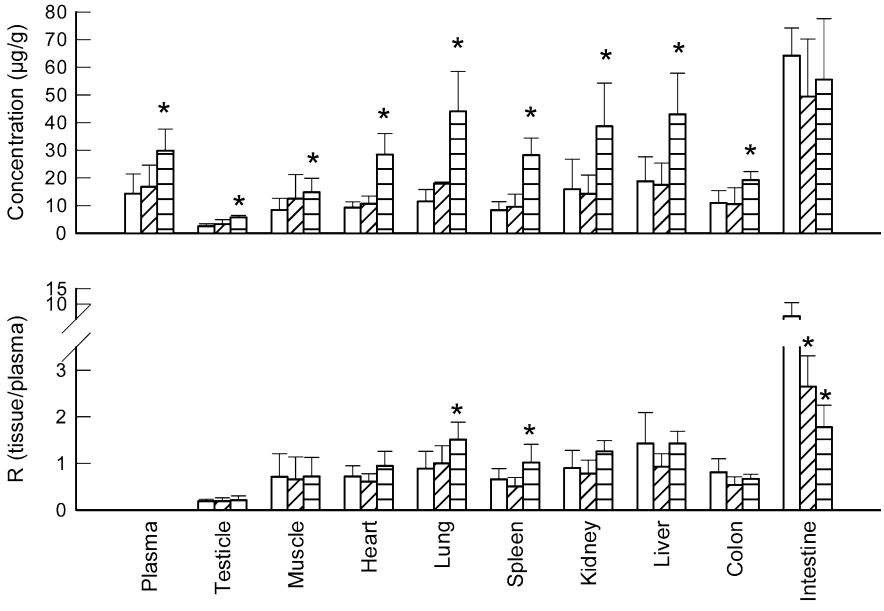

Fig. 3 *Top*: plasma and tissue concentrations (mean + SD) of etoposide determined after intravenous constant-rate infusion (10 mg/h) to rats coinfused with saline (*open bars*, control group), 1 mg/h of CyA (*diagonally hatched bars*, group 4) or 10 mg/h of CyA (*horizontally hatched bars*, group 5). *Bottom*: tissue-to-plasma concentration ratios of etoposide. *$P < 0.05$ vs corresponding control group value

27], with a decrease of intrinsic clearance as the dose increases, and saturable binding to many tissues. The results obtained in the present study using a constant-rate intravenous infusion of CyA (decrease of total blood clearance of CyA as the infusion rate increases) agree with the results obtained by Tanaka et al. [26, 27], and constitute an observable effect of these nonlinear factors, which are not seen when CyA is administered as an intravenous bolus.

Regarding the effect of CyA on the tissue distribution of etoposide under steady-state conditions, an increase in the tissue concentration of etoposide was observed in most of the tissues in rats of group 5 in comparison to the control group (Fig. 3), but this increase was due to the higher etoposide plasma concentration, and not to a direct effect of CyA on the distribution features of etoposide. In group 5, only lung and spleen showed tissue-to-plasma ratios of etoposide significantly higher than those obtained in the control group, although the increases in these ratios were low. In the case of intestine, the tissue-to-plasma ratio of etoposide was lower than that obtained in the control group. Similar results were obtained for the intestine when etoposide was intravenously administered as a bolus injection (group 2). This could have been due to the reported inhibition of biliary excretion of etoposide by CyA.

Human P-gp is present not only in tumor cells but also in normal tissues including the kidney, liver, small and large intestine, brain, testis, adrenal gland, and the pregnant uterus [7]. The expression of P-gp in the luminal epithelial cells of organs often associated with drug absorption and elimination, such as the intestinal mucosa, hepatocyte canalicular membrane, and renal proximal tubules, suggests that the physiological function of P-gp is protection of the organism, decreasing the absorption and increasing the elimination of toxic xenobiotics and/or metabolites. P-gp expression in other organs, such as the testis and endothelial cells comprising the blood–brain barrier, would protect specific organs as a consequence of the P-gp-mediated drug efflux from cells into the extracellular space. In the present study, the effect of CyA on etoposide distribution into the brain was not investigated. However, a previous study has shown that CyA does not increase the brain/blood ratio of etoposide [28]. The dose-limiting toxicity of etoposide is typically myelosuppression, and the affected cells express P-gp. Indeed, it has recently been reported that P-gp protects the bone marrow from vincristine-induced toxicity [29]. In the present study, the effect of CyA on etoposide distribution in bone marrow was not investigated due to methodological limitations, since only very small samples of this tissue can be obtained in rats.

It can be concluded that, when coadministering etoposide and CyA, steady-state blood concentrations of CyA higher than plasma concentrations of etoposide cause a decrease in the clearance of etoposide and, consequently, an increase in plasma and tissue concentrations. In our study, only lung and spleen tissues showed a tissue-to-plasma ratio of etoposide slightly increased by CyA. In the clinical practice, the coadministration of etoposide and CyA would not lead to an increase in toxicity of etoposide if plasma concentration of etoposide were maintained in the same range as when administered alone. This could be achieved by common pharmacokinetic methods such as decreasing the dose in the same proportion as clearance of etoposide is decreased by CyA administration.

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