ORIGINAL ARTICLE



Effects of cyclosporine A on the hepatobiliary disposition and hepatic uptake of etoposide in an isolated perfused rat liver model

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Abstract

Purpose A recirculating isolated perfused rat liver model was used to investigate the hepatobiliary disposition of etoposide and the effects of cyclosporine A (CyA) on the pattern of drug disposition in the bile and uptake in the liver.

Methods The portal vein, bile duct, and superior vena cava were cannulated in four groups of rats. The perfusions were conducted in the control group, which only received 10 μ g/ml etoposide, and the tested groups which received etoposide and CyA in 0.4, 2, and 10 mg/kg doses. Perfusate and bile samples were collected up to 180 min.

Results The determination of etoposide in the samples and homogenized liver by the high-performance liquid chromatography method showed that the administration of CyA led to significant changes in the hepatic excretion $(E_{\rm h})$, hepatic clearance $(CL_{\rm h})$, and half-life $(T_{1/2})$ of etoposide in the CyA 2 and 10 mg/kg treatment groups but not in 0.4 mg/kg group. The volume of the bile decreased to 64 and 45 % and biliary clearance $(CL_{\rm b})$ of etoposide reduced by 73 and 82 % in 0.4 and 2 mg/kg CyA group, respectively, when compared with the control group.

Conclusions These results demonstrated the dosedependant non-specific inhibitory effects of CyA on

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p-glycoproteins, multidrug resistance protein 2, bile salt export pump, and organic anion-transporting polypeptide, the drug transporters responsible for etoposide hepatobiliary disposition, hepatic uptake, and bile formation in rat.

Keywords Etoposide · Drug transporters · Hepatobiliary disposition · Hepatic uptake

Introduction

Drug transporters are proteins which have attracted widespread attention in recent years. These proteins play a significant role in many processes in pharmacokinetics and pharmacodynamics, including drug disposition and response. Organic cation transporters (OCTs), organic anion transporters (OATs), and organic anion-transporting polypeptides (OATPs) belong to the family of SLCs and play roles in the uptake of compounds into cells. The bile salt export pump (BSEP), p-glycoprotein (MDR), multidrug resistance proteins (MRPs), and breast cancer resistance proteins (BCRPs) belong to the family of ABCs, and their functions lead to the efflux of substrates from cells [1].

In the liver, the expression of these proteins leads to the uptake of substrates delivered through the portal vein from the basolateral membrane into hepatocytes and, then, their efflux into bile via the canalicular membrane [2].

ABCs can also be expressed in solid tumours and sometimes result in chemotherapeutic failure due to a decrease in the anticancer agent concentration in tumour cells. Considering the function of efflux transporters, it is obvious that the inhibition of these drug transporters by inhibitors such as cyclosporine A (CyA) may lead to better responses in cancer treatment [3, 4].

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Etoposide is an anticancer agent which is used in different malignancies such as lung cancer, testicular cancer, and leukaemia [5, 6]. Previous studies have shown that etoposide is a substrate in drug transporters, such as p-glycoproteins, MRPs, and BCRPs [7, 8]. The modulation of these proteins has been considered to achieve a more efficient chemotherapy response in some trials. In these trials, CyA was used in combination with etoposide, and area under the curve (AUC) elevations and changes in the pharmacokinetic parameters, such as clearance and elimination half-life in co-administration with CyA, have been reported. Also, CyA brought about hyperbilirubinemia in patients because of the inhibition of bilirubin transport in the liver which resolved after discontinuation of CyA [9, 10]. Despite the evaluation of the inhibitory effect of CyA on drug transporters, the extent of its influence on hepatobiliary disposition and the etoposide liver uptake has not been measured.

The aim of the present study was to clarify the effects of the co-administration of CyA in low, medium, and high doses with etoposide as an uptake and efflux transporter proteins inhibitor in an isolated perfused rat liver model (IPRL). The IPRL is an ex vivo model which is used to assess the specific role of the liver in the kinetics (uptake, metabolism, excretion) and the hepatobiliary disposition of drugs [11]. It has been hypothesized that CyA can lead to changes in the etoposide uptake of hepatocytes and disposition in the bile through drug transporter proteins.

Materials and methods

Chemicals and reagents

Etoposide was kindly provided by Cipla (Mumbai, India), and CyA, as Sandimmune 50 mg/ml (Pfizer Company), was used in this study. HPLC-grade solvents, including acetonitrile, methanol, chloroform, and n-hexane were obtained from Merck (Darmstadt, Germany), and all other chemicals and reagents were of analytical grade.

Liver perfusion

The male Sprague–Dawley rats used in this study were housed under constant conditions with a 12-h light/dark cycle and free access to food and water.

The rats were anaesthetized using a mixture of ketamine-xylazine (10–1 mg/kg), and then the liver was isolated for an ex vivo recirculating perfusion system. The portal vein, inferior vena cava (as input and output of the perfusion medium), and bile duct were cannulated.

The perfusion solution contained freshly prepared Krebs-Henseleit buffer (118 m MNaCl, 4.5 mM KCl, 2.75 mM CaCl2, 1.19 mM KH₂PO₄, 1.18 mM MgSO₄, and 25 mM NaHCO₃, equilibrated with 95 % O₂/5 % CO₂; pH 7.4). The total volume of the reservoir was 200 ml. Glucose (1 g/l) and etoposide (10 μ g/ml) were added to the perfusion solution before the experiment, and the perfusion solution was conducted by a flow rate of 10 ml/min. The temperature (37 °C), pH (7.4), and perfusion pressure (9 mmHg) were intermittently monitored and remained constant throughout the liver perfusion. Liver enzyme activities (ALAT and ASAT) and overall macroscopic appearance of the liver were continuously monitored during the perfusion period and used as a measure of liver viability.

Experimental design

To determine the effects of CyA on the hepatobiliary disposition of etoposide and the formation and excretion of bile, four different groups of rats were used, each containing six rats weighing between 250 and 300 g. The control group was perfused with etoposide only. The treatment groups consisted of high, medium, and low doses of CyA, and these groups received 10, 2, and 0.4 mg/kg body weight cyclosporine, respectively. The liver was first stabilized by the perfusion medium for 10 min; then, after the stabilization period, perfusion in the control group was continued with the etoposide-containing perfusion medium (10 µg/ ml). However, perfusion in the liver treatment groups was first continued by a 100-µl bolus injection of CyA into the portal vein catheter over 1 min; then, the etoposide-containing perfusion medium (10 µg/ml) was passed through the liver for 180 min.

Samples were taken until 180 min from the liver output at 10 min intervals and from the bile at 30 min intervals. All samples were centrifuged $(10,000 \times g, 10 \text{ min})$. At the end of the perfusion experiment, the livers were weighed and homogenized using normal saline (2:1 V/W), and all samples were frozen at -70 °C until assayed.

HPLC analysis of samples

Calibration curve for etoposide perfusion samples was drawn over the range from 0.05 to 50 µg/ml (0.05, 0.1, 0.4, 1, 10, 25, and 50 µg/ml).The chromatographic apparatus consisted of a low-pressure gradient HPLC pump and a UV detector (Knauer, Berlin, Germany). The data were acquired and processed using ChromGate chromatography software (Knauer, Berlin, Germany), and the HPLC method used for the analysis of the samples was previously described by Saadati et al. [12], with slight modifications. Briefly, the separation was performed in an RP-18, 150×4.6 mm column under isocratic elution; the mobile phase was a mixture of KH₂PO₄/acetonitrile/methanol (55:25:20, adjusted to pH 5.2) and pumped by a flow rate of 1 ml/min. The eluent was monitored at $\lambda = 285$ nm.

For the preparation of the liver samples, $200 \ \mu$ l of homogenized liver was mixed with a 1 ml mixture of chloroform and n-hexane (80:20, v/v) as the extraction solvent. After vortex mixing for 10 min and centrifugation ($10,000 \times g$, 10 min), the upper aqueous layer was discarded. The clear organic residue was transferred to a clean tube and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 120 μ l of the mobile phase and mixed well, and finally, 100 μ l of the clear solution was injected into the HPLC system. The perfusion and bile samples were directly injected into the HPLC system after centrifugation.

Pharmacokinetic analysis

In order to calculate the area under the perfusate concentration–time curves (AUC), the trapezoidal rule was used as described below:

$$AUC_{(0-t)} = (C_0 + C_t)(t)/2 \text{ and}$$

$$AUC_{(0-\infty)} = AUC_{(0-t)} + C_{last}/K$$
(1)

where C_{last} is the last concentration of perfusate at the end of the perfusion time and *K* is the perfusate elimination rate constant, which was estimated using the slope of the logarithm of the concentration–time curve.

The hepatic extraction ratio was defined as:

$$E_{\rm h} = (C_{\rm in} - C_{\rm out})/C_{\rm in} \tag{2}$$

The hepatic clearance of etoposide was determined using the following equation:

$$CL_{\rm h} = E_{\rm h} \times Q \tag{3}$$

where Q is the perfusion flow rate (10 ml/min).

The elimination half-life of etoposide was estimated according to the following:

$$T_{1/2} = 0.693/K \tag{4}$$

The percentages of the recovered etoposide doses in the perfusate, bile, and liver were calculated by multiplying the amount of etoposide in the perfusate, bile, or liver samples in 180 min, divided by the etoposide amount added to the perfusion medium.

Biliary clearance was estimated by dividing the total amount of the drug dose recovered in the bile over 180 min to the AUC₍₀₋₁₈₀₎ of the perfusate concentration–time curve.

The inhibition percentage of the etoposide biliary excretion due to the modulatory effect of CyA over 180 min was determined as:



Fig. 1 Perfusate concentration–time profiles of etoposide in logarithmic scale in the isolated perfused rat liver after addition of 10 μ /ml (2 mg) etoposide to the perfusion reservoir in the absence of any pretreatment (control) or after pretreatment with CyA 0.4, 2, and 10 mg/ kg (mean \pm SD)

The total amount of the recovered dose at the end of perfusion was estimated by adding the cumulative amount of the drug in the perfusate and bile, and the amount recovered in the liver.

Statistical analysis

The data were reported as the mean \pm SD, and the differences between the groups were analysed by SPSS 20, using the one-way ANOVA followed by Tukey's post hoc test.

Results

The perfusate concentration-time profile after the liver perfusion of the perfusion medium containing etoposide in a dose of 10 µg/ml in a logarithmic scale is displayed (Fig. 1). This graph shows a rapid decline in the etoposide perfusate concentration during the first 90-min period and a slight decrease after the second 90-min period. The control and CyA low-dose groups had approximately equal trends in the perfusate concentration changes, which decreased to 50 % of the administered dose of etoposide over 180 min. However, in the medium and high doses (2, 10 mg/kg), the perfusate concentration decreased to 65 and 88 % of the administered etoposide dose, respectively.

Table 1 compares the hepatic extraction ratio, hepatic clearance, and elimination half-life of etoposide in the control and CyA treatment groups. It can be clearly seen that $E_{\rm h}$ is roughly equal in the control and CyA low-dose groups (0.54 \pm 0.038 and 0.49 \pm 0.051). However, a

 $\%I = \frac{\text{Total amount of eto in bile in control group} - \text{Total amount of eto in bile in CyA group} \times 100$

Total amount of eto in bile in control group

Table 1 Hepatic disposition parameters of etoposide in isolated perfused rat liver after addition of 10 μ /ml (2 mg) etoposide to the perfusion reservoir in the absence of any pretreatment (control) or after pretreatment with CyA 0.4, 2, and 10 mg/kg (mean \pm SD)

Group	E _h	CL _h (ml/min)	T _{1/2} (min)
Control	0.54 ± 0.038^{a}	5.49 ± 0.38^{a}	31.93 ± 8.63^{a}
CyA, 0.4 mg/kg	0.49 ± 0.051^a	4.95 ± 0.51^{a}	42.75 ± 5.84^a
CyA, 2 mg/kg	$0.35\pm0.075^{\text{b}}$	$3.53\pm0.75^{\text{b}}$	61.62 ± 18.07^{b}
CyA, 10 mg/kg	$0.12\pm0.061^{\rm c}$	$1.23\pm0.61^{\rm c}$	$149.5 \pm 29.67^{\circ}$

Means that are significantly different from each other (p < 0.05) are shown with different letters (a, b, c)

Table 2 Volume of bile, biliary clearance of etoposide, and percentage of inhibition effect of CyA on biliary clearance of etoposide in isolated perfused rat liver after addition of 10 μ /ml (2 mg) etoposide to the perfusion reservoir in the absence of any pretreatment (control) or after pretreatment with CyA 0.4, 2, and 10 mg/kg (mean \pm SD)

Group	Volume of bile (µl)	CL _b (µl/min)	%I
Control	2250.83 ± 390.04^{a}	309.27 ± 41.39^{a}	0
CyA, 0.4 mg/kg	$1410.83 \pm 90.08^{\rm b}$	$82.85\pm14.56^{\text{b}}$	73.27
CyA, 2 mg/kg	$1000.66 \pm 90.91^{\circ}$	$55.51\pm8.71^{\text{b}}$	82.06
CyA, 10 mg/kg	0^d	0^{c}	100

Means that are significantly different from each other (p < 0.05) are shown with different letters (a, b, c)

significant difference is observed between the calculated $E_{\rm h}$ in the CyA medium- and high-dose groups (0.35 \pm 0.075 and 0.12 \pm 0.061, respectively) and control group. It is worth noting that the difference between the $E_{\rm h}$ in the CyA medium- and high-dose groups is also significant. The same results were observed for the $CL_{\rm h}$ and $T_{1/2}$ in the study groups (Table 1).

The data relating to the bile samples are shown in Table 2 and Figs. 2 and 3. According to Table 2, the volume of the secreted bile (V_b) and biliary clearance (CL_b) of etoposide were reduced by the increase in the CvA dose, with a meaningful difference in these parameters between the groups. In comparison with the control group, the V_b decreased to 64 % in the low-dose group and 45 % in the medium-dose group. Similarly, CL_b was markedly reduced by 73 and 82 % in the low- and medium-dose groups, compared to the control group. As seen in Table 2, both the V_b and CL_b decreased to zero in the CyA high-dose group. The percentage of inhibition of the etoposide biliary excretion is shown in Table 2, and it is clear from this table that the inhibitory effect of CyA rose to 73 and 82 % in the 0.4 and 2 mg/kg doses, compared to the control group. A complete inhibition in the etoposide biliary excretion was observed in the high-dose CyA group. Figure 2 shows a plot of the mean \pm SD cumulative etoposide biliary excretion (µg) versus time (min) for the effect of CyA when



Fig. 2 Cumulative amount of etoposide excreted in bile in the isolated perfused rat liver after addition of 10 μ /ml (2 mg) etoposide to the perfusion reservoir in the absence of any pretreatment (control) or after pretreatment with CyA 0.4, 2, and 10 mg/kg (mean \pm SD)

co-administered with etoposide. The cumulative biliary excretion of etoposide was reduced by 72 and 80 % in the CyA low- and medium-dose groups.

The percentage of the recovered dose in the liver (a), the liver/perfusate concentration ratio (b), and the bile/liver concentration ratio (c) is displayed in Fig. 3. This figure shows that the percentage of the dose of etoposide recovered from the liver is equal in the control group and the low- and medium-dose CyA groups and is approximately 6.5 % of the administered dose. However, the dose of etoposide recovered from the liver in the high-dose group was reduced to 5 % of the administered dose.

Figure 3b shows the liver/perfusate concentration ratio, where the CyA in all of the applied doses has actually reduced the liver uptake of the drug compared to the control group.

Figure 3c demonstrates the bile/liver concentration ratio of etoposide, and this graph shows that this ratio is equal in the 0.4 and 2 mg/kg CyA groups and is approximately 4 times less than in the control group.

The recovery of etoposide from the bile, liver, and perfusate and the total recovered dose are presented (Fig. 4). It is worth mentioning that the total recovered dose of etoposide is almost the same as in the control, low-dose and medium-dose CyA groups, but it was 20 % greater in the CyA high-dose group.

Measurement of the hepatic enzymes using commercially available kits showed a slight raise for ALAT and ASAT activity during the perfusion period in control, 0.4, and 2 mg/kg doses of CyA groups (Fig. 5). However, the hepatic enzymes activity increased with a higher slope in 10 mg/kg CyA group (Fig. 5).

Discussion

There are several clinical trial studies that are focused on various effects of CyA on pharmacokinetic behaviour of



Fig. 3 Recovered dose in liver (a), the liver/perfusate concentration ratio (b), and the bile/liver concentration ratio(c) of etoposide in the isolated perfused rat livers after addition of 10 μ /ml (2 mg) etoposide to the perfusion reservoir in the absence of any pretreatment (control) or after pretreatment with CyA 0.4, 2, and 10 mg/kg (mean \pm SD). Means that are significantly different from each other show with different letters (p < 0.05)

etoposide [13–15]. Although they all pointed that CyA can cause a significant decrease in total clearance of etoposide, there is a report which shows that the mentioned decrease in etoposide clearance is mainly due to non-renal mechanisms [15]. So far, there is no report relating to the exact effect of CyA on hepatic clearance. The design of this research makes it possible to evaluate the etoposide interactions with different transporters and its hepatic disposition during administration of etoposide alone and its co-administration with different doses of CyA as a modulator of efflux transporters in isolated perfused liver of rats.

First of all, the viability of liver is discussed here because the liver should be alive and functional during the perfusion. As mentioned before, in this experiment, viability of the liver was confirmed by the overall normal macroscopic appearance and the wet weight of the livers which was <4 % of body weight at the end of the perfusion. The other marker which could be used to evaluate the viability of the liver is the hepatic enzyme level (ALAT & ASAT). As seen in Fig. 5a and b, the concentration of ALAT or ASAT in the perfusate samples increased slightly during the perfusion, but remained in the acceptable range. However, the pattern of increase in ALAT and ASAT was almost the same in the control, low- and medium-dose CyA groups. As well as in the 10 mg/kg CyA group, in first 90-min period, the aminotransferases level was respectable for the normal hepatocytes function (Fig. 5). The enzymes level during the second 90-min period in this group can be considered as an indicator of hepatic damage. There are two factors possibly inducing hepatic damages in this experiment: the perfusion system itself and the CyA, which is a hepatotoxic drug. The perfusion system was approved beforehand and can keep the liver alive during 180 min of the perfusion time [16].

The hepatotoxicity and inhibitory effects of CyA on drug transporters have been shown to be dose dependent. Previous studies have shown that different doses of CyA led to various effects on bile flow and the hepatic enzymes level. Based on the previously performed experiments [17–19], CyA in the doses up to 14 mg/l could not lead to any liver damages. Therefore, it has been assumed that the 0.4, 2, and 10 mg/kg doses of CyA (approximately equal to, respectively, 0.5, 2.5, and 12.5 mg/l) could not result in the liver shutting down. Moreover, there is a study which reported that the hepatobiliary disposition of MTX, in co-administration with CyA, did not change in a group which received only a CyA formulation vehicle (Cremophor EL and ethanol) compared to the control group [19]. Consequently, it was concluded that the mentioned effects entirely depend on the CyA itself and not its vehicles. Assuming that, the perfusion processes itself and CyA-containing medium could not induce a liver damage (shown by ALAT and ASAT). One can suppose that etoposide due to its hepatotoxicity effects can potentiate the hepatotoxic effects of CyA in co-administration of CyA and etoposide, which may lead to full inhibition of transporters in charge of bile formation and/or secretion in high-dose CyA group. The mentioned evidence pointed to the conclusion that the low and medium levels of CyA (resulted in concentrations of 500 and 2500 ng/ml) would be safer, which is in agreement with the results of a trial study where the steady state Fig. 4 Percent of recovered dose of etoposide in the perfusate, bile, liver, and total recovered dose in the isolated perfused rat livers after addition of 10 μ /ml (2 mg) etoposide to the perfusion reservoir in the absence of any pretreatment (control) or after pretreatment with CyA 0.4, 2, and 10 mg/kg (mean \pm SD). Means that are significantly different from each other show with different letters (*p* < 0.05)

a

ALAT Activity (UIL)

\$0

60

40

20

0

30

60 90



Fig. 5 Alanine aminotransferase, ALAT, (a) and aspartate aminotransferase, ASAT, (b) in the perfusate samples in control or after pretreatment with CyA 0.4, 2, and 10 mg/kg groups (mean \pm SD). In

Time (min)

120 150

this measurement method, the normal range for ALAT is (0-46 U/l) and for ASAT is (0-49 U/l)

CyA levels up to 4800 ng/ml were considered to be safe in combination with etoposide [13].

Etoposide is a well-known substrate of several drug transporters. One previous study on wild-type and mutant mice showed that the hepatobiliary disposition of etoposide is mostly carried out by MRP_2 and that P-glycoprotein is the main transporter for the excretion of the drug into the intestines [7].

Real drug efflux in the bile (bile/liver concentration ratio) is estimated from the amount of drug uptake into the hepatocytes and its excretion in the bile through the canalicular membrane. Figure 3c shows that the bile/liver concentration ratio was equal in the CyA low- and medium-dose groups, but different from the control group.

As seen in Table 2, the volume of bile during the perfusion study was reduced by 38 % in the low, 55 % in the medium, and 100 % in the high CyA dose groups. However, the etoposide biliary clearance, as mentioned before, was relatively equal in the low- and medium-dose CyA groups and was five times less than in the control group. It can be concluded that the inhibitory effect of CyA on MRP₂, the main transporter responsible for etoposide disposition in bile, in the 0.4 and 2 mg/kg doses was approximately equal. However, this inhibitory effect on BSEP, the protein which produced a considerable amount of bile, was markedly different for the three different doses of CyA,

leading to a significantly different volume of bile. Previous studies have shown that CyA in low doses has a less inhibitory effect on MRP₂ when compared to P-glycoprotein. For example, a study reported that a 10 μ m CyA solution has a complete inhibitory effect on P-glycoprotein, but the same concentration inhibits MRP₂ activity by just 25 % [20]. However, this partial inhibitory effect of low CyA doses can be potentiated by increasing the CyA dose, and the high amount of CyA can lead to the full inhibition of MRP₂. Similarly, the data obtained from this study suggested that a 10 mg/kg dose of CyA caused the complete inhibition of bile secretions and etoposide biliary clearance.

The etoposide perfusate concentration, as seen in Fig. 1, decreased in the control and CyA-treated groups because of binding to liver tissue and the secretion in the bile. The rate of decline was more rapid during the early time points of the study and then slowed down in the control, low and medium doses of CyA until the final time point. However, in the CyA high-dose group, the etoposide concentration increased slightly in the last 90 min of the experiment, which could possibly be due to the release of etoposide from its binding sites in the liver tissue [17].

The hepatic disposition parameters (E_h , CL_h , $T_{1/2}$), as seen in Table 1, were nearly equal in the control and CyA low-dose groups. Regarding the 0.4 mg/kg dose of CyA, approximately 50 % of the administered etoposide was extracted by the liver, which was not significantly different from the control group. However, this parameter was markedly changed in the other groups, leading to a 35 and 12 %hepatic extraction in the 2 and 10 mg/kg doses of CyA, which were significantly different from the control group. Moreover, hepatic clearance of etoposide decreased about 35 and 77 % in CyA medium- and high-dose groups when compared to the control and CyA low-dose groups, which is in agreement with the result of Lum et al.'s study. They reported a 46 % decrease in clearance of etoposide during CyA treatment (greater than 2000 ng/ml) in adults [14]. Similar to the Lum et al.'s report, the effect of the CyA on the hepatic disposition parameters had a dose-dependent manner in our experiment that was much greater in the high doses of the modulator. The mentioned decrease in total clearance (the previous studies) and hepatic clearance (the present study) leads to a significant increase in etoposide concentration, suggesting a main decrease in etoposide dose in order to ensure equal exposure.

The etoposide liver uptake, as seen in Fig. 3a, was reduced in the high-dose CyA group when compared to the other groups. Considering the variation in the perfusate concentration in different groups, the correct drug liver uptake can be estimated by the liver/perfusate ratio, as seen in Fig. 3c. Compared to the control group, a 30 % reduction in the uptake of the drug by the liver was observed for the low dose of CyA (0.4 mg/kg) and the medium dose of CyA (2 mg/kg), and a 54 % reduction was observed for the high dose of CyA (10 mg/kg). Organic anion-transporting polypeptides (OATPs) are the main transporters responsible for the hepatocyte drug uptake, and previous studies have shown the role of MRP₃ as an efflux pump for etoposide in the sinusoidal membrane of hepatocytes [7, 21, 22].

One study was designed to evaluate the effects of fooddrug interactions on OATP1b1, OATP1a1, and OATP2b1. A 50 % reduction in the bioavailability of etoposide because of the inhibition of OATPs by grapefruit juice was reported [23]. So far, there is no other research about sinusoidal transporters and their roles in the liver uptake of etoposide. Some evidence shows that the OATP response to the inhibitory effect of CyA is dose dependent [24, 25]. The reduction in the liver uptake of etoposide by the increase in CyA in our research, which is also dose dependent, may prove that OATPs play a dominant role in the drug entrance into hepatocytes. The determination of specific subfamily transporters that are responsible for the liver uptake of etoposide requires additional molecular experiments.

In conclusion, it is proved that the CyA led to significant and considerable changes in the hepatobiliary parameters of etoposide in a dose-dependant manner. The changes in the hepatic clearance of etoposide observed in this study should be considered to adjust the dose of etoposide in coadministration with CyA in clinical trial studies. **Acknowledgments** This study was part of a bio-pharmaceutical and pharmacological thesis supported by Tehran University of Medical Sciences.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the Animal Ethic Committee and Institutional Review Board of Pharmaceutical Research Centre of Tehran University of Medical Sciences.

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