Effects of P-glycoprotein Modulators on Etoposide Elimination and Central Nervous System Distribution

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Accepted for publication July 23, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
In this study, P-glycoprotein modulator effects on pharmacokinetics and central nervous system distribution of the chemotherapeutic agent etoposide were evaluated. The multidrug resistance transporter P-glycoprotein is expressed in normal tissues, and its physiological function is thought to be an excretory and/or protective one. To examine this further, we evaluated etoposide under steady-state and bolus dose conditions. In microdialysis infusion studies, etoposide 15 mg/kg/hr was administered to 12 rats. Rats received sodium cyanide (1 or 100 mM), trifluoperazine (30 mM) or cyclosporine (4.14 mM) via microdialysis probe at 3.5 hr after etoposide infusion initiation. High-dose sodium cyanide (100 mM) increased the etoposide BBRcorr from 0.09 ± 0.03 to 0.85 ± 0.35. Similarly, trifluoperazine significantly increased the BBRcorr (0.05 ± 0.02 vs. 1.30 ± 0.43), whereas cyclosporine had no effect. In bolus studies, etoposide (10–12 mg/kg) was given alone or concomitant to cyclosporine (5 mg/kg) or tamoxifen (13.5 mg/kg). Control etoposide total systemic clearance (ml/min/kg) was 29.3 ± 13.0 vs. 16.0 ± 1.9 and 22.6 ± 5.3 for cyclosporine and tamoxifen treatments, respectively. Etoposide nonrenal clearance (ml/min/kg) values for cyclosporine (12.0 ± 1.6) and tamoxifen (18.1 ± 3.6) treatments was also decreased from controls (23.5 ± 10.5). Etoposide renal clearance (ml/min/kg) values (5.7 ± 2.5) were not significantly different from cyclosporine (4.0 ± 0.7) or tamoxifen (4.6 ± 1.7) treatments, respectively. In this study, the ability of sodium cyanide and trifluoperazine to alter etoposide BBRcorr, demonstrated that etoposide distribution into brain is partly controlled by an active transport process. Similarly, the results indicate cyclosporine inhibits etoposide transport at the canalicular membrane and/or etoposide P-450 metabolism.

The multidrug resistance phenomenon includes cross-resistance among the naturally derived cancer agents, including the anthracyclines (doxorubicin, daunorubicin), epipodophyllotoxins (etoposide, teniposide), vinca alkaloids (vinblastine, vincristine), taxanes (taxol, taxotere) and various other lipophilic compounds (Lum et al., 1993). Although these compounds differ structurally, they all are large lipophilic, naturally occurring compounds that contain complex ring structures. This drug resistance is associated with the MDRI gene, which encodes for a protein known as P-glycoprotein (160–180 kDa). This protein has been shown to act as an energy-dependent efflux pump which transports substrates out of the cell (before cytotoxicity occurs). Not only has P-glycoprotein been implicated in multidrug resistance, but also recent literature suggests that P-glycoprotein almost certainly has a physiological function in normal tissues relating to the excretion and/or protection of tissues from naturally occurring toxins or xenobiotics (Burgio et al., 1996; Colombo et al., 1994; Speeg et al., 1992; Lum et al., 1992). Recently P-glycoprotein has been shown to be expressed in several normal tissues, including the CNS, kidney, liver and colon (Lum et al., 1993; Cordon-Cardo et al., 1989; Hegmann et al., 1992; Jette et al., 1993; Thiebaut et al., 1989; Liberman et al., 1989; Thiebaut et al., 1987). More specifically, increased levels were found at the renal proximal tubule in the kidney (Liberman et al., 1989), the biliary canalicular membrane of the liver (Thiebaut et al., 1987) and the endothelial cells at the BBB and blood-testes barrier (Cordon-Cardo et al., 1989; Hegmann et al., 1992). Expression of this membrane bound protein at these localized regions in the kidney and liver suggests a secretory role of P-glycoprotein. Alternatively, the expression at the BBB suggests that P-glycoprotein serves as a pump to eliminate toxic or potentially harmful compounds from the CNS. Clinical studies have demonstrated that the addition of a P-glycoprotein modulator to antineoplastic dosing regimens results in an increased incidence of CNS toxicity and in some cases death (Lum et al., 1992).

ABBREVIATIONS: BBB, blood-brain barrier; CNS, central nervous system.
These studies implicate P-glycoprotein as having a functional involvement in the efflux of antineoplastic agents out of the CNS, limiting the toxicity of these compounds resulting in lower concentrations in the brain compared with the blood. Studies have further shown that concentrations of various antineoplastic agents can be up to 50 times lower in the CNS compared with that of the plasma compartment (Levin 1986). These lower levels may be due to the expression of P-glycoprotein at the BBB, which may actively remove these agents from the CNS.

In rodents, there are two different drug-transporting P-glycoproteins that seem to function in a manner similar to that of the human MDR1 P-glycoprotein. These proteins are designated as mdr1a and mdr1b. The mdr1a and mdr1b P-glycoproteins are largely overlapping but do not have identical drug transport capabilities. The mdr1a has been found predominantly in the intestine, liver, brain and testis. Additionally, mdr1b is substantially present in adrenal, placenta, ovarian and uterus. In the kidney, both the mdr1a and mdr1b are present in similar amounts (Borst et al., 1994, 1993). Mice homozygous for disruption of the mdr1a gene have been shown to be sensitive to the toxic effects of P-glycoprotein substrates. Disruption of the mouse mdr1a P-glycoprotein (knockout mouse) resulted in an increased sensitivity to the centrally neurotoxic pesticide ivermectin (100-fold) and also to the antineoplastic drug vinblastine (3-fold) (Schinkel et al., 1994). These animals also showed profound alterations in pharmacokinetics and tissue distribution when compared with controls, although detailed CNS distribution studies were not performed. Later, studies utilizing this same model showed that doxorubicin and cyclosporine in brain tissues were significantly higher (35- and 17-fold) at 4 hr after intravenous injection than that of the wild-type mice that expressed mdr1a (Schinkel et al., 1994). Similarly, in 1995, it has been demonstrated (Burgio et al., 1996) that cyclosporine is a potent inhibitor of etoposide clearance in the rat animal model. Similarly, it was also shown that etosposide had a brain-blood ratio of <1, giving evidence that etosposide distribution into the CNS is controlled, in part, by a mechanism other than passive diffusion (i.e., active transport). This current evidence has led to the present study in which the objective was to (1) evaluate if the concentration of the cytotoxic agent etosposide in the CNS is controlled, in part, by an active transporter (possibly P-glycoprotein) and (2) evaluate the mechanism (renal or nonrenal) by which the modulators cyclosporine and tamoxifen inhibit etosposide clearance.

Materials and Methods

Chemicals. Etoposide (Vepesid) and cyclosporine (Sandimmune) were obtained from Amerisource (Louisville, KY); etoposide (VM-16), tamoxifen hydrochloride, trifluoperazine dihydrochloride, citric acid (anhydrous), benzyl alcohol, polyoxyethylene-sorbitan monooleate (Tween 80) and polyethylene glycol 300 were purchased from Sigma Chemical (St. Louis, MO); teniposide (VM-26) was a gift from the Bristol-Myers Squibb (Syracuse, NY); sodium cyanide and alcohol 95% were obtained from Fisher Scientific (Fair Lawn, NJ); and antipyrine was obtained from Ransdell (Louisville, KY).

Animals. Adult male Sprague-Dawley rats weighing between 275 and 388 g were obtained from Harlan Laboratories (Indianapolis, IN) and used in all in vivo experiments. Animals were maintained under a 12:12-hr light/dark cycle and had access to food and water ad libitum before the studies.

Drug administration and sampling protocol (pharmacokinetic study). Utilizing i.v. bolus dose conditions, the pharmacokinetics of etoposide alone (10–12 mg/kg) and in the presence of cyclosporine (5 mg/kg) or tamoxifen (13.5 mg/kg) were evaluated. A total of 15 animals were used, with 5 randomized to each of the three groups. In each group, vehicle (control) or modulator was given to 20 to 30 min before giving etoposide. Etoposide dosing solution was prepared by diluting commercial vepesid with 0.9% sodium chloride 1:1 (10 mg/ml) on the day of the experiment. Cyclosporine dosing solution was made by diluting commercial Sandimmun (50 mg/ml) with a blank dosing vehicle consisting of citric acid (400 mg), benzyl alcohol (6 g), polyisorbate 80 (16 g), polyethylene glycol 300 (130 g) and 95% alcohol (61 ml) to obtain a concentration of 10 mg/ml. This solution was subsequently diluted with 0.9% sodium chloride 1:1 to obtain a final concentration of 5 mg/ml. Tamoxifen hydrochloride was reconstituted in the same blank dosing vehicle (27 mg/ml) and diluted with 0.9% sodium chloride 1:1 to obtain a concentration of 13.5 mg/ml.

Animals were cannulated in both the femoral vein and artery for serial blood sampling and drug administration, respectively. Animals were allowed to recover for ~24 hr before initiating the experiment. Multiple blood samples were obtained for 8 hr (0, 0.17, 0.5, 1, 2, 3, 4, 5, 6 and 8) via the femoral vein. These represent approximate sampling times; actual sampling times were noted and used in the pharmacokinetic analysis. To enable separation of renal and nonrenal clearances, animals were housed in metabolic cages, and urine was collected for 24 hr.

Drug administration and sampling protocol (microdialysis study). Etoposide drug solution was prepared by diluting commercial vepesid (20 mg/ml etoposide) 1:1 with 0.9% NaCl. In these studies, animals were maintained under anesthesia throughout the experiment, and two microdialysis probes were implanted into the CNS. Commercial CMA/12 and CMA/20 microdialysis probes were used. In the CNS, one probe was placed in the left frontal cortex (treatment), and the other was placed in the right (control), with the coordinates of 3.0 mm lateral and 3.0 mm anterior to bregma for each site. This was to ensure maximum distance between the probes placed into the left and right frontal cortices. Probe insertion was accomplished by a Kopf stereotaxic frame, lowering the probes at a rate of 0.5 mm/30 sec. After probe implantation was complete, probes were allowed to equilibrate in the CNS for 1 hr before starting the experiment. In these experiments, etoposide was administered as an i.v. infusion (15 mg/kg/hr) to 12 adult male Sprague-Dawley rats for 7 hr. Also, rats received an etosposide i.v. bolus of 15 mg before starting the infusion. Rats also received either 4.14 mM cyclosporine (n = 3), 30 mM trifluoperazine hydrochloride (n = 3) or 1 mM or 100 mM sodium cyanide (n = 6) 3.5 hr after initiating the etosposide infusion in the dialysate perfusing the left microdialysis probe.

In vivo microdialysis. An artificial cerebral spinal fluid (pH 7.35) was prepared containing (in mM) Na+ 155; K+ 2.9; Ca2+ 2; Mg2+ 0.7; Cl− 138; HCO3−; and glucose 6.0. In all experiments delivery of the microdialysate was accomplished by a microinjection pump (CMA/100; Carnegie Medicin) at 2 μl/min. Dialysate samples were taken every 30 min. Commercial probes (4 mm in length) were used in the CNS (CMA/12; Carnegie Medicin) and jugular vein (CMA/20 soft probe; Carnegie Medicin). All surgeries were performed under anesthesia with ketamine (85 mg/kg i.p.) and acepromazine (1.6 mg/kg i.p.). Anesthesia was maintained with periodic intramuscular injections of this combination as needed. Body temperature was maintained at 37°C with an electric heating pad checked via a rectal thermometer.

Retrodialysis. Relative etosposide microdialysis probe in vivo recovery was estimated using antipyrine as a retrodialysis marker. Etoposide extent of distribution into the CNS (BBRcorr) was calculated by dividing corrected brain dialysate concentrations by corrected jugular dialysate concentrations for both the left and right frontal cortices. The usefulness of this approach has been demon-
strated previously (Burgio and McNamara 1993a, 1993b; Burgio et al., 1996).

**Assay methodology.** Etoposide was analyzed by modification of the HPLC method described by Stiff et al. (1992). Dialysate samples were analyzed by a specialized microbore HPLC system that allows determination of small sample volumes, such as 2 to 5 μl. Chromatography was performed on an HPLC component system consisting of a CMA/200 microsampler, FL-45 detector and a PM-80 pump (Bioanalytical Systems, West Lafayette, IN). The mobile phase consisted of 10 μM ammonium acetate buffer, pH 5.0 to 5.5/methanol (54:46), and the effluent was monitored by fluorescence detection at Ex 230 nm and Em 325 nm, respectively. Separations were carried out on a C18 (5 μm particle size) 100 × 1.0 mm column (Bioanalytical Systems) with a flow rate of 0.1 ml/min. Analytical sensitivity was 15 ng/ml with a 5-μl injection (70 pg on column). In vivo dialysate samples were injected directly onto the HPLC column without an extraction. In the rat serum analysis, 100 μl of serum was placed into a glass culture tube, and 25 μl of internal standard (Teniposide 20 μg/ml) was added. Samples were extracted with 1 ml of methylene chloride by vortexing for 30 sec. After centrifugation at 1800 g for 7 min, the organic layer was transferred and evaporated to dryness under nitrogen at room temperature. The residue was reconstituted in 100 μl of mobile phase. The sample was then vortexed for 30 sec, and an aliquot of 5 μl was injected onto the HPLC system.

Antipyrine was quantified by HPLC via a modification of Brouwer et al. (1984). The mobile phase consisted of 0.6 M potassium phosphate/acetonitrile (75:25), with 1 ml of triethylamine added to each liter. The effluent was monitored by UV detection at 254 nm. Separations were carried out on a C18 analytical column and a C18 guard column, with a flow rate of 1 ml/min at room temperature. Antipyrine dialysate samples were injected directly onto the HPLC column without an extraction.

**Data analysis.** In the bolus dose studies, etoposide total drug concentration vs. time data were analyzed by fitting a monoeponential or biexponential equation to these profiles using nonlinear regression analysis (Rstrip; MicroMath, Salt Lake city, UT). The area under the drug concentration-time curve (AUC) and area under the first moment curve (AUMC) were determined from the coefficients and exponents of these fitted relationships. Total systemic clearance was determined from: CLs = dose/AUC, where dose and AUC, are the dose of etoposide administered to the animal and area under the drug concentration-time curve in serum. The renal clearance was calculated via CLr = Xu8/AUCs, where Xu8 refers to the total amount of unchanged drug excreted in the urine.

The Clnr (clearance nonrenal) was determined by subtracting the renal clearance from the total systemic clearance: Clnr = Cls – CLr.

The steady-state volume of distribution was determined from the equation: Vss = dose × AUMC/AUC2.

The BBR,corr (brain-blood ratio corrected) was calculated by: BBR,corr = brain [ET]corr/jugular [ET]corr, where brain [ET]corr and jugular [ET]corr are the etoposide concentrations corrected (by antipyrine) as determined from the microdialysis probes in the left and right frontal cortices and the jugular vein.

**Statistics.** In the bolus dose studies, a one-way ANOVA followed by a Fisher protected least-significant difference test were used to assess differences between the three groups (etoposide, etoposide and cyclosporine, etoposide and tamoxifen). An a priori level of significance was set at P < .05. In the microdialysis experiments, comparisons of the animals in which two probes were implanted, a two-way ANOVA (repeated measures) followed by a Duncan’s post hoc test was used to determine if a treatment was significantly different from control. Again, an a priori level of significance was set at P < .05.

**Results**

**Local administration (CNS).** Table 1 summarizes the results of the 1- and 100-mM dose of sodium cyanide as it perfused through the microdialysis probe on the BBR,corr of etoposide. The low dose of sodium cyanide (1 mM) did not alter the etoposide BBR,corr (0.13 ± 0.11 vs. 0.14 ± 0.09). However, the higher dose (100 mM) of sodium cyanide re-

| TABLE 1 | Brain-blood corrected ratios of etoposide following local administration (CNS) of sodium cyanide (1 and 100 mM), trifluoperazine (30 mM) and cyclosporine (4.14 mM) treatment in the left (treatment) and right (control) frontal cortex |
|---------|-------------------------------------------------|-------------------------------------------------|
| **Animal** | **Left** | **Post** | **Right** |
| **BBR,corr** | | | |
| Sodium cyanide (1 mM) | | | |
| 51 | 0.26 | 0.24 | 0.02 | 0.04 |
| 52 | 0.08 | 0.12 | 0.04 | 0.02 |
| 53 | 0.05 | 0.06 | 0.02 | 0.05 |
| Mean (± S.D.) | 0.13 (0.11) | 0.14 (0.09) | 0.03 (0.01) | 0.04 (0.02) |
| Sodium cyanide (100 mM) | | | |
| 55 | 0.09 | 1.05 | 0.04 | 0.07 |
| 56 | 0.06 | 0.45 | 0.05 | 0.05 |
| 57 | 0.12 | 1.06 | 0.07 | 0.05 |
| Mean (± S.D.) | 0.09 (0.03) | 0.85* (0.35) | 0.05 (0.02) | 0.06 (0.01) |
| Trifluoperazine (30 mM) | | | |
| 60 | 0.26 | 1.20 | 0.06 | 0.3 |
| 61 | 0.07 | 1.88 | 0.03 | 0.06 |
| 62 | 0.05 | 1.08 | 0.08 | 0.06 |
| Mean (± S.D.) | 0.05 (0.02) | 1.39* (0.43) | 0.06 | 0.05 |
| Cyclosporine (4.14 mM) | | | |
| 64 | 0.09 | 0.09 | 0.07 | 0.10 |
| 65 | 0.07 | 0.20 | 0.05 | 0.15 |
| 66 | 0.07 | 0.23 | 0.13 | 0.22 |
| Mean (± S.D.) | 0.08 (0.01) | 0.17 (0.07) | 0.08 (0.04) | 0.16 (0.06) |

* Statistically significant, P < .05.
sulted in a significant increase in the etoposide BBR$_{corr}$ (0.09 ± 0.03 vs. 0.85 ± 0.35). Figure 1 shows that after the addition of sodium cyanide (100 mM) to the left frontal cortex there was a dramatic increase in the etoposide BBR$_{corr}$ (P < .05) while no affect on the right BBR$_{corr}$.

To further demonstrate that etoposide may be extruded from the brain extracellular fluid by P-glycoprotein, the P-glycoprotein modulator trifluoperazine was given via the microdialysis probe as in the sodium cyanide experiment. The results of adding 30 mM trifluoperazine to the microdialysate are given in table 1. The dose of trifluoperazine (30 mM) was show to significantly alter the BBR$_{corr}$ of etoposide (0.05 ± 0.02 pretreatment vs. 1.39 ± 0.43 post-treatment). Figure 2 shows a plot of the mean (±S.E.M.) BBR$_{corr}$ vs. time for etoposide after treatment with trifluoperazine. Figure 2 demonstrates that trifluoperazine (30 mM) resulted in the BBR$_{corr}$ increasing to a value similar to unity.

Treatment with cyclosporine (4 mM) was shown not to increase the BBR$_{corr}$ of etoposide (0.08 ± 0.01 vs. 0.17 ± 0.07), although there was a slight trend for an increase (table 1 and fig. 3). This was probably due to the presence of the cremophor EL, which had to be added to the dialysate to permit the solubilization of cyclosporine. The lipophilic nature of the cremophor probably increased the sequestration of etoposide out of the surrounding tissues. However, this was appropriately controlled by the right frontal cortex, which only had the cyclosporine vehicle (i.e., cremophor EL). As shown in figure 3, there was no statistical difference in the BBR$_{corr}$ between the right frontal cortex (control) and the left frontal cortex (treatment).

**Systemic administration.** In figure 4, the pharmacokinetic profiles for the three treatment groups are shown (mean data). In controls, etoposide total systemic clearance (ml/min/kg) was 29.3 ± 13.0 vs. 16.0 ± 1.9 and 22.6 ± 5.3 for cyclosporine and tamoxifen treatments, respectively (table 2). Similarly, nonrenal clearance (ml/min/kg) values for cyclosporine (12.0 ± 1.6) and tamoxifen (18.1 ± 3.6) were also decreased from controls (25.5 ± 10.5). However, the renal clearance (ml/min/kg) values for controls (5.7 ± 2.5) were not significantly different from treatments, cyclosporine (4.0 ± 0.7) or tamoxifen (4.6 ± 1.7). Similarly etoposide volume of distribution was unchanged in the presence of cyclosporine or tamoxifen (table 2). The pharmacokinetic profile for etoposide after tamoxifen treatment was similar to the control (etoposide alone). However, this was not the case for the cyclosporine treated animals. The biphasic portion (i.e., distribution phase) of the curve was less distinct on the addition of cyclosporine. Overall, total systemic clearance (ml/min/kg) of etoposide was decreased (29.3 ± 13.0 vs. 16.0 ± 1.9) (table 2).

**Discussion**

Previous studies in our laboratory have shown that etoposide has a BBR$_{corr}$ of <1 (Burgio et al., 1996). This suggested that a transport mechanism might be involved at the BBB retarding the permeation of etoposide into the CNS. To fur-
TABLE 2
Mean pharmacokinetic parameters of etoposide alone, etoposide (with tamoxifen) and etoposide (with cyclosporine)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Etoposide</th>
<th>ET + TAM</th>
<th>ET + CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL_\text{a} (ml/min/kg)</td>
<td>29.3 (13.0)</td>
<td>22.6 (5.3)</td>
<td>16.0 (1.9)</td>
</tr>
<tr>
<td>CL_\text{w} (ml/min/kg)</td>
<td>23.5 (10.5)</td>
<td>18.1 (3.6)</td>
<td>12.9 (1.6)</td>
</tr>
<tr>
<td>Cl (ml/min/kg)</td>
<td>5.7 (2.5)</td>
<td>4.8 (1.7)</td>
<td>4.8 (0.7)</td>
</tr>
<tr>
<td>V_{ss} (ml/kg)</td>
<td>2877 (1586)</td>
<td>1764 (739)</td>
<td>1217 (424)</td>
</tr>
</tbody>
</table>

Mean ($\pm$ S.D.), n = 5 per group.
* P < .05 (one-way ANOVA, Fisher).

ther investigate this possibility, a group of animals was used to examine the effect of sodium cyanide on the BBR of etoposide. Previous studies have shown that ATP-dependent transport mechanisms in vitro and in vivo can be inhibited by a variety of metabolic inhibitors, including sodium cyanide, 2,4-dinitrophenol and sodium azide (Allen et al., 1995; Hori et al., 1993; Tsuji et al., 1993). In the current study, only the high dose (100 mM) sodium cyanide was able to modulate the BBR of etoposide (table 1). The rationale for the failure of the lower dose of sodium cyanide to inhibit the efflux of etoposide may be three related reasons. First, the amount of sodium cyanide that actually is present at the site of the probe is much lower than 1 mM because the loss of drug from the microdialysis probe is not 100%. Second, as the distance (radius) increases from the probe, the concentration of sodium cyanide would decrease proportionally within the surrounding tissues. Thus, the location of the transporter(s) in relation to the placement of the microdialysis probe is critical, if an effect is to be seen. In point, the greater the distance from the site of the probe implantation, the higher the dose (concentration) that would be needed to alter an ATP-dependent mechanism. Third, the number (capacity) of transporters present may be so large that the lower dose of cyanide cannot elicit an effect (i.e., the lower dose is unable to inhibit all the transporters present in the tissue).

As mentioned, the addition of the high dose of sodium cyanide resulted in a significant increase in the BBR of etoposide (fig. 1). This inhibition resulted in a BBR of ~1, suggesting that etoposide transport out of the brain extracellular fluid was totally inhibited. This study along with previous data (etoposide BBR, corr of 1 at steady state) supports the notion that etoposide is actively transported out of the CNS and can be inhibited locally by a metabolic inhibitor such as sodium cyanide. To further suggest that P-glycoprotein was involved in the efflux of etoposide at the CNS, trifluoperazine was used in a separate group of animals. Calmodulin inhibitors such as trifluoperazine have been demonstrated to be capable of significantly sensitizing MDR cells in vitro (Ganapathi and Grabowski, 1983; Ganapathi et al., 1991); however, its usefulness in vivo has yet to be demonstrated. In cell lines in which expression of P-glycoprotein was correlated with the level of resistance, trifluoperazine (5 μM) was shown to potentiate the cytotoxicity of vincristine by 2- to 7-fold (Ganapathi et al., 1991). In another study, Tsuruo et al. (1982) demonstrated that trifluoperazine, at nontoxic concentrations, resulted in a 10-fold increase in doxorubicin sensitivity in resistant murine cells and a 5-fold increase in doxorubicin accumulation. This was despite the fact that trifluoperazine did not significantly alter drug cytotoxicity or accumulation in sensitive cell lines. This suggests that trifluoperazine can modulate P-glycoprotein without being toxic to normal cells. Similarly, our data show that the P-glycoprotein modulator trifluoperazine (30 mM) could inhibit etoposide transport out of the extracellular fluid as supported by the increase in the BBR of etoposide at steady state in the rat animal model (fig. 2).

Although cyclosporine has been demonstrated to be a potent inhibitor of P-glycoprotein (Tamai and Safa, 1990), it was not shown to alter the BBR of etoposide at the dose used (via CNS administration). This may be due to the fact that the concentration of cyclosporine was insufficient to garner an effect. The concentration of cyclosporine (4.14 mM) may have been too low (similar to the 1 mM sodium cyanide), but due to the limited solubility of cyclosporine, the molar concentration could not be increased without altering the dialysate. Also contributing to the lack of effect could be the extremely low recovery and loss exhibited by cyclosporine with microdialysis probes. Elmquist and Yang (1993) showed that large lipophilic compounds like cyclosporin A tended to adhere to the membrane of the microdialysis probe, thereby reducing both loss and recovery. It was also suggested that there is self-association between the cyclosporine molecules, again leading to a decrease in the availability of cyclosporine as it progressed through the microdialysis probe. Thus, in these experiments, the overall amount of cyclosporine that had an opportunity to elicit an effect may be very low.

In the bolus dose study, the cyclosporine-treated animals showed a dramatic decrease in systemic clearance (table 2). These data support earlier work by Keller et al. (1992) where they observed the effects of the cyclosporine analog PSC-833 on etoposide pharmacokinetics. In the Wistar rat, it was demonstrated that PSC-833 (5 mg/kg) prolonged the distribution half-life of etoposide from 18 to 48 min. This resulted in a >50% decrease in the systemic clearance of etoposide. However, renal and nonrenal mechanisms were not differentiated in this particular study. In the present study, on average, there was a ~50% reduction in etoposide clearance on the addition of cyclosporine (5 mg/kg) (table 2). These data were similar to the earlier work using infusion studies with etoposide (Burgio et al., 1996). Here it was demonstrated that upon the addition of cyclosporine 5 mg/kg, the variability in the systemic clearance (ml/min/kg) pretreatment of etoposide (CV = 44%) was significantly reduced after treatment (CV = 12%). This evidence along with previous infusion studies (data not shown) suggests that cyclosporine inhibits the variable portion of etoposide clearance in the rat. In our previous studies (Burgio et al., 1996), we demonstrated considerable interanimal variation in the unbound clearance of etoposide, with the percent change in unbound clearance highly correlated with the pretreatment clearance, suggesting that the higher the pretreatment clearance value for etoposide, the greater the potential for an interaction with cyclosporine. Table 2 also shows that cyclosporine inhibition of etoposide
clearance was mainly due to a nonrenal mechanism. Neither cyclosporine nor tamoxifen treatment was significantly different compared with controls with regard to renal clearance.

On average, only 20% of the dose was excreted unchanged in the urine after 24 hr. This is in contrast to human studies that demonstrate that renal elimination (up to as much as 50%) plays a major role in the excretion of etoposide (Hande, 1992; Clark and Slevin, 1987; Arbuck et al., 1986). Human studies have indicated a correlation between etoposide clearance and creatinine clearance and that dose reductions might be appropriate in patients with impared renal function (Arbuck et al., 1986). There was no change statistically in etoposide renal clearance after cyclosporine or tamoxifen treatment in the rat. Similarly, the volume of distribution at steady state was unchanged between the treatment groups and controls, although there was a trend for a decrease after treatment with cyclosporine.

Currently, the nonrenal elimination fate of the etoposide dose is poorly understood. In this study, nonrenal elimination is responsible for >50% of etoposide’s clearance. This is even greater than that reported in humans (Lum et al., 1992; Hande, 1992). In the present study cyclosporine inhibited the nonrenal clearance of etoposide by ~50%. Two possibilities exist for this interaction. First, a site that has potential for cyclosporine modulation is that of P-glycoprotein-mediated drug transport. P-glycoprotein has been shown to be highly expressed at the canalicular membranes of the liver (Thiebaut et al., 1987). Similarly, in vitro and in vivo studies have demonstrated that ~50% to 70% of etoposide is excreted as unchanged drug into the bile (Hande et al., 1988a, 1988b; Savoca and Gaver, 1989). In the isolated perfused liver model in the rat, it was shown that >90% of the etoposide drug clearance could be explained by direct biliary clearance of etoposide or conjugation of the glucuronide. In support of the previous work, Savoca and Gaver (1989) demonstrated that 74% and 26% of the administered 14C etoposide dose was recovered in the feces and urine, respectively.

The second potential site for cyclosporine alteration of etoposide clearance could be the inhibition of drug metabolism. In vitro, using isolated rat perfused livers, studies indicate that etoposide glucuronide is a major metabolite of etoposide (Hande et al., 1988a, 1988b). Similarly, other studies using rat and mouse liver microsomal preparations have demonstrated metabolic activation of etoposide via cytochrome P-450-mediated demethylation reactions (Van Maaneen et al., 1987; Haim et al., 1987). Relling et al. (1992) demonstrated that etoposide catechol and ethoxy coumarin O-deethylating activities were detectable in all human livers studied (n = 26). However, the formation of etoposide catechol in these studies only represented a very small portion of the total drug that was placed into the microsomal preparation. Earlier studies, using human liver microsomes, also indicated a weak interaction existed between etoposide and the mephentoin hydroxylase P-450 enzyme (Relling et al., 1989). Because cyclosporine has been shown to undergo P-450 metabolism via hydroxylation and demethylation, the possible interaction with etoposide through inhibition of metabolism cannot be totally discounted.

In the current study, it was shown that etoposide elimination was decreased by 50% on the addition of cyclosporine. This decrease in etoposide clearance was mainly due to a nonrenal mechanism. These data along with the current literature purport that cyclosporine has its effects on etoposide clearance (in the rat) mostly through P-glycoprotein-mediated drug transport at the canalicular membrane in the liver and/or inhibition of drug metabolism. Also, these data along with our previous studies (Burgio et al., 1996) indicate that the variability associated with etoposide elimination in these animals is sensitive to cyclosporine, suggesting that expression of P-glycoprotein at various sites in the eliminating organs, such as the liver and/or kidney, may be dramatically different within a given animal population. This may help explain the variable results (i.e., toxicities) in human populations when a P-glycoprotein modulator is given concomitantly with a neoplastic agent. Similarly, it is possible that drug-metabolizing capabilities are highly variable. However, evidence for this is limited.

In conclusion, etoposide distribution into the brain appears to be controlled in part by an active transport process (probably P-glycoprotein). This conclusion is supported by the observation that etoposide has a BBRcorr of much less than 1 and can be inhibited by a metabolic inhibitor such as sodium cyanide and a P-glycoprotein substrate (trifluoperazine).

References


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