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Inhibition of intestinal P-glycoprotein and effects on etoposide absorption

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Abstract P-glycoprotein (Pgp) actively pumps a number of antineoplastic drugs, such as etoposide, out of cancer cells and causes multidrug resistance. Pgp is also expressed at the brush-border membrane of the small intestine under normal physiological conditions. We hypothesized that inhibition of intestinal Pgp might decrease the efflux of etoposide from the blood into the intestinal lumen, thereby, increasing the bioavailability of etoposide. The absorption of etoposide was studied using everted gut sacs prepared from rat jejunum and ileum. The addition of C219, a monoclonal antibody of Pgp, at 100 ng/ml or of 0.2 *M* 5'-adenylylimidodiphosphate, a nonhydrolyzable adenosine triphosphate (ATP) analog, increased the absorption of etoposide. Quinidine, an antiarrythmic agent, has been demonstrated to circumvent multidrug resistance in cell lines, possibly by interfering with Pgp function. Adding quinidine at 1 mg/ml to the everted gut sac increased the absorption of etoposide. In vivo absorption of etoposide was also studied by intraluminal perfusion of the drug in the small intestine of anesthetized rats. Intravenous infusion of quinidine at either 1 or 2 mg/h increased the serum level of etoposide in a dose-dependent manner. Intravenous infusion of etoposide at 0.2 mg/h resulted in luminal exsorption of the drug in the small intestine. The intestinal clearance of etoposide was 41.7 ± 7.2 ml kg^{-1}, which decreased to 18.4 ± 3.9 ml kg^{-1} with the infusion of quinidine at 1 mg/h. The present data confirm that intestinal Pgp mediates the efflux of etoposide and that the use of Pgp-inhibiting agents such as quinidine may increase the bioavailability of etoposide.

Key words: Etoposide · Quinidine · P-glycoprotein Multidrug resistance · ABC proteins

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Introduction

P-glycoprotein (Pgp) actively extrudes a number of anticancer drugs, such as etoposide, from cancer cells [17, 18]. Cells exposed to a single anticancer drug may develop resistance to a broad range of structurally and functionally unrelated drugs due to expression of Pgp. The phenomenon known as one of the common mechanisms for multidrug resistance (MDR). Under normal physiological conditions, Pgp may also be expressed in various organs, such as the lung, kidney, liver, adrenal tissues, pancreas, and colon as well as in the brush border membrane of the small intestine [4, 6, 7, 12, 20]. However, the physiological function of Pgp is not yet known.

Pharmacological agents antagonizing multidrug resistance (MDR-reversing agents) include a wide variety of compounds [8]. These agents probably block Pgp and increase cytotoxicity of anticancer drugs. MDR-reversing agents may also affect the pharmacokinetics of anticancer drugs. Among the MDR-reversing agents, amiodarone [11], cyclosporine [16], and SDZ PSC 833 [15] not only affect the drug resistance of cancer cells but also alter the pharmacokinetics of etoposide. Previous reports showed a decreased clearance and a prolonged half-life of etoposide when MDR-reversing agents were used simultaneously [11, 15, 16].

Etoposide tablets are used clinically in oral anticancer therapy. Pgp located at the brush-border membrane may pump out orally absorbed etoposide and, hence, decrease the drug's bioavailability. We hypothesized that the induction of intestinal Pgp might increase etoposide efflux from the blood into the intestinal lumen, consequently decreasing the drug's absorption. The inhibition of intestinal Pgp might therefore increase the bioavailability of etoposide. The effect of the intestinal Pgp level on the absorption of anti-cancer drugs is of great experimental interest and clinical importance. In this paper we report that etoposide

absorption in the everted gut sacs of rats can be modulated by inhibition of Pgp in the small intestine. Cinchona alkaloids, including quinidine, are well known as a MDR-reversing agents [22]. The effects of quinidine on the absorption and exsorption of etoposide in the whole animal were also investigated.

Materials and methods

Materials

Male Sprague-Dawley rats bred and housed in the animal center of National Cheng Kung University, Medical College, were used. Food was withheld for 1 day before the experiment. Water was freely available. For in vivo studies, rats were anesthetized with urethane (1 g/kg, intraperitoneal injection). Etoposide and teniposide were supplied by Bristol-Myers Co. (Evansville, Ind.). Quinidine sulfate and 5'-adenylylimidodiphosphate (AMPPNP) were obtained from Sigma Chemical Co. (St. Louis, Mo). Most other chemical reagents were purchased from E. Merck (Darmstadt, Germany). All chemicals used were of the highest purity available. The monoclonal antibody of Pgp, C219, and its control negative antibody were obtained from Centocor Diagnostics, Inc. (Malvern, Pa). Tyrode solution was prepared by dissolving 24 g NaCl (137 m*M*), 3 g dextrose (5.6 m*M*), 3 g $NaHCO_3$ (12 m*M*), 6 ml 10% Kcl (2.7 m*M*), 7.8 ml 10% $MgSO_4 \cdot 7H_2O$ (1.1 m*M*), 3.9 ml 5% $NaH_2PO_4 \cdot 2H_2O$ (0.42 m*M*), and 5.4 ml 1 *M* $CaCl_2$ (1.8 m*M*) in 3 l water.

Etoposide assay

Etoposide was analyzed by a high-performance liquid chromatographic (HPLC) method modified from a previous report [3]. In brief, 200 μl serum or intestinal perfusate was mixed with 20 μl of teniposide (10 μg/μl in methanol), the internal standard. The mixture was extracted with 2 ml chloroform. A 1.5 ml volume of the organic phase was evaporated by nitrogen gas and then reconstituted with 200 μl mobile phase, and 30 μl was injected onto the HPLC column. The HPLC system consisted of a Waters 600E pump equipped with a satellite 700 WISP automated injector (Millipore Co., Milford, Mass.), a μ-Bondapack C18 column (30-cm length, 3.9-mm inside diameter, Millipore Co.), and a fluorescence detector (Kratos 980; Applied Biosystems Inc., Ramsey, NJ) . The mobile phase included 45% methanol and 0.1% acetic acid run at a flow rate of 2 ml/min. The eluent was monitored with an excitation wavelength of 215 nm and an emission wavelength of 280 nm. The retention times of etoposide and teniposide were 4.8 and 12.4 min, respectively. The ratio of etoposide to teniposide by peak height was compared with the calibration curve for quantitation.

Absorption of etoposide in everted gut sacs

The jejunum and distal ileum of the rat intestine (approx. 25 cm each) were taken and everted sacs were prepared using the method developed by Wilson and Wiseman [21]. Six sacs were filled with 3 ml Tyrode's solution and placed in 50 ml Tyrode's solution containing 100 μg etoposide/ml at 37°C. In all, 200 μl of the solution inside the sacs was taken every 10 min. In the inhibition studies, either 1 mg quinidine/ml (a Pgp inhibitor), 100 ng C219/ml (a monoclonal antibody of Pgp), or 0.2 *M* of AMPPNP [a nonhydrolyzable adenosine triphosphate (ATP) analog] was pretreated for 30 min before the addition of etoposide. The negative antibody of C219 was also used at 100 ng/ml in a separate experiment as an additional control.

Absorption of etoposide in vivo

An in vivo recirculated-perfusion technique similar to that employed in exsorption studies was used [13]. The jugular vein was cannulated with silastic tubing (0.5-mm inside diameter, 0.95-mm outside diameter; Dow Corning Co., Midland, Mich.) and was infused with saline as the control or with quinidine sulfate solution (1 or 2 mg/ml) at 1.02 ml/h. The beginning of the duodenum and the end of ileum were intubated with Teflon tubing (3-mm inside diameter 4 mm outside diameter) and both were connected to a peristaltic pump. The loop was circulated with 100 ml Tyrode's solution containing 0.1 mg etoposide/ml. Blood samples (0.5 ml each) were taken via the carotid artery cannula. Blood was allowed to clot and serum was separated for HPLC analysis.

Exsorption of etoposide in vivo

An in vivo single-pass perfusion technique was used [13]. Etoposide (0.2 mg/ml) and quinidine (0 or 1 mg/ml) were infused through the jugular vein at 1.02 ml/h. The bile duct was ligated and diverted with Tygon tubing (0.25-mm inside diameter, 0.76-mm outside diameter; Norton/Chemplast, Wayne, N.J.). Blood samples were collected via the carotid artery cannula. The intestinal segment was perfused at a flow rate of approximately 20 ml/h. The intestinal perfusate was collected hourly. The intestinal clearance of etoposide was calculated by dividing the rate of excretion by the mean drug concentration measured in the serum [13].

Results

Inhibition of etoposide absorption in everted gut sacs

Etoposide was transported from the mucosal side (bulk solution) to the serosal side (inside sac) after a lag period of approximately 10 min at different segments of the small intestine. When Pgp inhibitors were added, the concentrations of etoposide in the sacs increased, indicating a decrease in etoposide efflux. Etoposide absorption was facilitated by the addition of monoclonal antibody C219 (Fig. 1). The negative antibody of C219 also showed a nonspecific inhibitory effect on etoposide efflux. The etoposide concentration measured in sacs pretreated with monoclonal antibody C219 was significantly different from that detected in sacs pretreated with the control negative antibody after 40 min in both the ileum and the jejunum ($P < 0.05$, $n = 6$ animals in each group). The specific inhibitory effect of C219, shown as the difference between C219 and its negative antibody, was more pronounced in the ileum than in the jejunum. Similarly, AMPPNP or quinidine inhibited etoposide efflux and increased etoposide absorption (Fig. 2). The etoposide concentration measured in sacs pretreated with quinidine or AMPPNP was significantly different from that detected in control sacs after 40 min in both ileum and jejunum ($P < 0.05$, $n = 6$ animals in each group). The inhibitory effect of quinidine was approximately the same in the ileum and the jejunum. AMPPNP, on the other hand, showed a higher inhibitory effect in the jejunum than in the ileum.

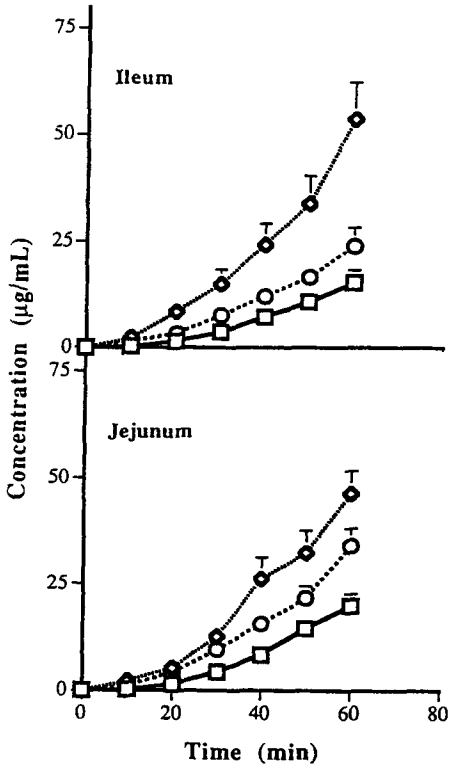

Fig. 1 The time profile of etoposide concentration inside the everted intestinal sacs of rats ($n = 6$ animals in each group). The *lower panel* illustrates data from the jejunum and the *upper panel*, data from the distal ileum. At either site, the monoclonal antibody C219 (◇) or its negative antibody (○) increased the absorption of etoposide. The etoposide concentration measured inside the sacs was higher in both treatment groups than in the control group (□).

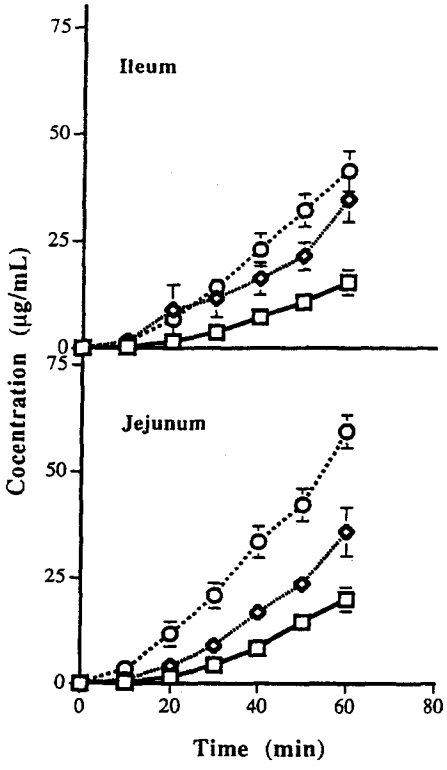

Fig. 2 The time profile of etoposide concentration inside the everted intestinal sacs of rats ($n = 6$ animals in each group). The *lower panel* illustrates data from the jejunum and the upper panel, data from the distal ileum. At either site, quinidine (◇) and AMPPNP (○) increased the rate of etoposide absorption. The etoposide concentration measured inside the sacs was higher in drug-treated intestines than in the control group (□).

Absorption of etoposide in situ

When etoposide solution was circulated in the intestinal loop, the drug was slowly absorbed, its concentration in serum elevating gradually over the experimental period (Fig. 3). In rats intravascularly infused with quinidine at the same time, the serum concentrations of etoposide became significantly higher than did those in rats infused with normal saline ($P < 0.05$ for all time points, $n = 6$ animals in each group). The increase was dependent on the dose rate of quinidine infusion. After 6 h, the serum etoposide concentration increased by factors of 3 and 8 in rats infused with quinidine at 1 and 2 mg/h, respectively.

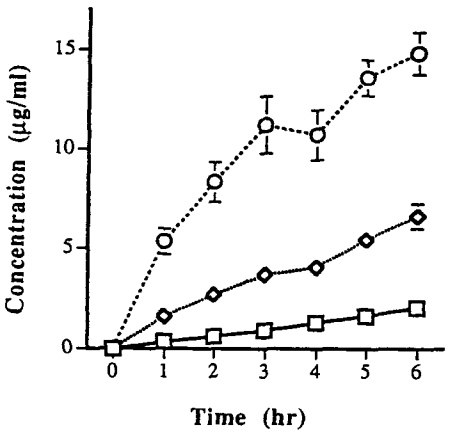

Fig. 3 The time profile of etoposide concentration in the serum of rats given etoposide intraluminally. Simultaneous quinidine infusion increased the etoposide serum concentration. (□, Control; (◇, 1-mg/h quinidine infusion; ○: 2-mg/h infusion of quinidine ($n = 6$ animals in each group).

Exsorption of etoposide in situ

When rats were infused with etoposide, the serum concentration gradually approached the steady state after 4 h of infusion. The mean serum concentration and the clearance were determined on the basis of the data obtained during the last 3-h period. The mean serum concentration of etoposide was 1.60 ± 0.34 μg/ml as determined in rats without the use of quinidine ($n = 7$), whereas a concentration of 2.29 ± 0.20 μg/ml was obtained with the co-administration of quinidine ($n = 8$). Quinidine infusion decreased both the intestinal clearance

and the total clearance of etoposide. The total clearance was 276 ± 28 and 170 ± 16 ml $h^{-1} kg^{-1}$ in control and quinidine-treated rats, respectively. The intestinal clearance was 41.7 ± 7.2 and 18.4 ± 3.8 ml $h^{-1} kg^{-1}$ in control and quinidine-treated rats, respectively. Moreover, the difference observed in total clearance or intestinal clearance between the control and the quinidine-treated rats was statistically significant ($P < 0.05$).

Discussion

The in vitro data support the hypothesis that etoposide is exsorbed by Pgp after its absorption in the small intestine. When Pgp was inhibited by the substrate inhibitor (quinidine), the unhydrolyzable ATP analog (AMPPNP), or the monoclonal antibody of Pgp (C219), etoposide exsorption was decreased and its absorption could be substantially increased. The use of MDR-reversing agents with anticancer drugs may not only enhance the cytotoxicity of the latter pharmacodynamically but also increase their absorption pharmacokinetically.

Monoclonal antibody C219 was used to inhibit etoposide efflux in the everted gut sacs by its placement in the luminal side of the membrane. C219 recognizes a highly conserved ATP-binding site found in all Pgp isoforms [9]. Interestingly, etoposide efflux was inhibited in spite of the ATP-binding site being considered to be located at the cytosolic side. Possible reasons for this inhibition would be that C219 monoclonal antibody might have been transported into the cytosol by some unknown mechanism or that the ATP-binding site of Pgp may not be located at the cytosolic side as predicted [10].

Etoposide has an oral bioavailability of about 10% in rats [19]. An increased serum concentration of etoposide is possibly due to an increased absorption, or a decreased elimination of the drug. Quinidine infusion (1 mg/h) decreased the total clearance of etoposide from 276 ± 28 to 170 ± 16 ml $h^{-1} kg^{-1}$, indicating a 38% decrease in elimination. In the absorption study, the etoposide concentration in serum increased more than 2-fold at the same rate of quinidine infusion. This suggests that quinidine not only decreased the rate of etoposide elimination but also increased the rate of etoposide absorption.

Both the total and the intestinal clearance of etoposide was decreased by the infusion of quinidine. The change noted in intestinal clearance (~ 24 ml $h^{-1} kg^{-1}$) was too small to explain the magnitude of change observed in total clearance (~ 100 ml $h^{-1} kg^{-1}$). Apparently, quinidine had inhibited the other pathways of etoposide elimination. Quinidine may have inhibited the metabolic enzyme in the liver or the tubular secretion of etoposide in the kidney. Besides, it is possible that Pgp in the liver and the kidney might also be involved in the elimination of etoposide. Quinidine may have inhibited Pgp in the liver and the kidney in a way similar to that repeated in the small intestine [5].

We have previously studied the effect of quinidine on the intestinal microclimate pH and the intestinal clearance of other drugs [2] and have concluded that there is a carrier-mediated process of drug exsorption that can be inhibited by quinidine. Because quinidine is a inhibitor of Pgp, a possible role of Pgp in intestinal drug exsorption was therefore proposed. The present study demonstrates that Pgp plays an important role in the intestinal exsorption of etoposide. The high intestinal clearance of some compounds [13] can be caused by Pgp transport in addition to the contributory effects of the unstirred water layer and the microclimate pH on the intestinal luminal surface, as we have previously proposed [1, 14].

The present study demonstrates that quinidine may increase etoposide absorption as well as inhibiting etoposide elimination at a concentration (< 1 μg/ml) below its therapeutic range [2]. Etoposide is a drug of low bioavailability. The combined use of etoposide with MDR-reversing agents such as quinidine will increase etoposide's bioavailability and the potency of the etoposide oral tablet. In addition, quinidine may also potentiate etoposide's action by inhibiting the occurrence of MDR in cancer cells. Further studies are worth pursuing to ensure the clinical benefit of the combination of these two drugs.

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