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Preclinical report

Effect of hydroxyzine on the transport of etoposide in rat small intestine

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Etoposide, an anti-neoplastic agent and a substrate of P-glycoprotein (P-gp), exhibits variable oral bioavailability. P-gp, the multidrug resistance gene (*mdr*1) product, has been considered as an absorption barrier against intestinal drug absorption. Terfenadine, an antihistamine, has been shown to be a P-gp inhibitor. The current study was designed to assess the effect of hydroxyzine, an antihistamine, on the transport of etoposide in the small intestine. Everted rat gut sacs were used to determine the absorption and exsorption of etoposide under different conditions, as rhodamine 123 was chosen to evaluate the role of P-gp in the drug interaction. The results showed that the transport of etoposide was significantly increased from the luminal site to the serosal site in the jejunum by 2- and 4-fold after 90 min in the presence of hydroxyzine and quinidine, respectively. A similar trend was observed in the ileal sacs. This *in vitro* exsorption study also demonstrated that hydroxyzine could reduce the efflux of etoposide to the luminal site in either jejunum or ileum. The effect of hydroxyzine on the pharmacokinetics of etoposide differed by the *in vivo* route of administration, thus assuming clinical importance for chemotherapeutic treatment. [© 2001 Lippincott Williams & Wilkins.]

Key words: Absorption, etoposide, hydroxyzine, small intestine.

Introduction

Etoposide, a podophyllotoxin derivative, is an anti-neoplastic agent that acts via inhibition of DNA topoisomerase II activity. It is commonly used in the treatment of neoplastic diseases such as small-cell lung cancer and Kaposi's sarcoma.[1,2] This anticancer drug exhibits various oral bioavailabilities with a range of 25–75%, and considerable intra- and interpatient variation.[1] It has been proved that etoposide is a P-glycoprotein (P-gp) substrate.[3,4]

P-gp, the gene product of *mdr*1, a 170-kDa plasma protein, functions as an energy-dependent drug efflux pump to decrease drug accumulation in a variety of systems.[5–7] P-gp is expressed under physiological conditions in a wide range of tissues, including the small intestine and colon. This membrane protein has been considered as an absorption barrier against intestinal drug absorption. An MDR-reversing agent can overcome the barrier and increase drug absorption. Several chemicals such as verapamil, cyclosporine A and PSC 833 have been proved to be potent P-gp inhibitors *in vitro*, but their toxicities have hindered their use in clinical application.[8]

A case report in 1992 mentioned that unexpected neutropenia occurred in a patient receiving doxorubicin and terfenadine.[9] A further study confirmed that terfenadine could restore sensitivity to MCF-1/ADR and L1210/VMDRC.06 cells.[10] Another study by Hu and Robert showed the P-gp-inhibitory effects of other antihistamines, azelastine and flezelastine, on the resistance to doxorubicin in C6 cells.[11] Several investigations have shown that the MDR-reversing agents share common characteristics such as a hydrophobic aromatic ring, a hydrophilic *N*-alkyl group, a vague 3-D structural similarity and a tertiary nitrogen positively charged at physiological pH levels.[12] There is also a similarity in the chemical structure between hydroxyzine and terfenadine (Figure 1), with the aromatic ring and a hydrophilic *N*-alkyl group. Since hydroxyzine is an antihistamine used in allergy, sedation, nausea and vomiting, it was judged to be interesting to evaluate the feasibility of hydroxyzine

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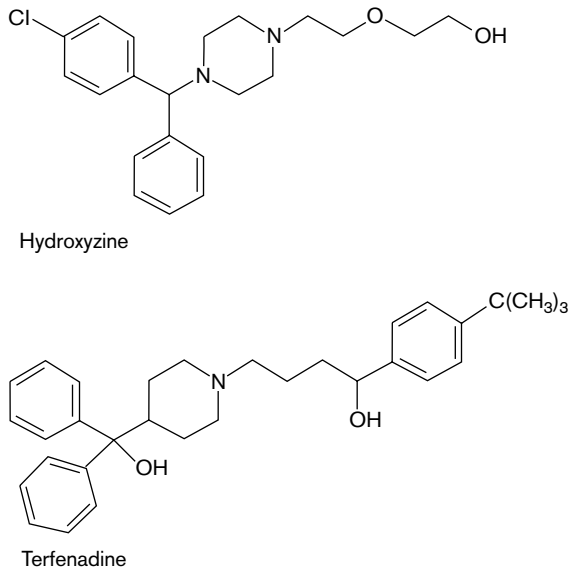

Figure 1. Chemical structures of terfenadine and hydroxyzine.

to overcome drug resistance and its influence on the absorption of etoposide.

In this study, an *in vitro* everted sac study was performed to evaluate the effect of hydroxyzine on the transport of etoposide in rat small intestine. Rhodamine 123 (RH123) was chosen as a substrate to investigate the role of P-gp in the drug interaction. Additionally, the effect of hydroxyzine on the plasma concentration of etoposide *in vivo* was assessed by i.v. infusion or intraluminal administration of etoposide. It is clinically important to understand the interaction between etoposide and anithistamines for the treatment of drug resistance.

Materials and methods

Chemicals

Male Sprague-Dawley rats bred and housed in the animal center of National Cheng Kung University, Medical College fasted overnight before the experiment. Water was given *ad libitum*. Etoposide was from Bristol-Myers Squibb (Princeton, NJ). Quinidine sulfate, hydroxyzine dihydrochloride, RH123 and urethane were purchased from Sigma (St Louis, MO). Tissue culture medium (TC199) was purchased from Gibco/BRL (Grand Island, NY). $CaCl_2$, NaH_2PO_4, NaCl, glucose, $NaHCO_3$, KCl and $MgSO_4 \cdot 7H_2O$ were obtained from Merck (Darmstadt, Germany). Methanol was purchased from BDH (Poole, UK). Dichloromethane was purchased from Mallinckrodt (Paris, KT). All chemicals were either analytical or high-performance liquid chromatography (HPLC) grade. Only deionized water was used.

Everted sac absorption study

Male Sprague-Dawley rats were sacrificed with ether and a 25-cm length of either jejunum or ileum was removed from 2–3 cm below the ligament of Treitz or 5 cm above the cecum, respectively. The segment was everted, ligated at both ends and filled with 3 ml of tissue culture medium (TC199). Subsequently, this sac was placed in 50 ml of TC199 containing 100 μg/ml of etoposide. The medium was gassed with air at 37°C. Two hundred microliters of samples inside the sacs was taken every 10 min up to 90 min.

Effect of P-gp inhibitors

Different concentrations of inhibitors were added outside the sacs for various pre-incubation times: 1 mg/ml quinidine for 30 min and 500 μM hydroxyzine for 20 min followed by the previously described procedure.

Everted sac exsorption study

A concentration of 100 μg/ml etoposide with different inhibitors in TC199 was added to the sacs and was gassed with air at 37°C. Different concentrations of inhibitors were added outside the sacs for various pre-incubation times. Five hundred microliters of samples was taken outside the sac every 10 min up to 90 min.

Everted sac exsorption study of RH123

Preparation of everted gut sacs and the procedure of exsorption study were the same as described previously. A concentration of 20 μg/m of RH123 was added to the sacs. One milliliter of sample outside the sac was taken every 30 min up to 90 min. The concentration of RH123 was measured by a spectrofluorophotometer (RF-1501; Shimadzu, Kyoto, Japan) at λ_{ex} at 485 nm and λ_{em} at 546 nm.

Intestinal exsorption of etoposide *in vivo*

To evaluate the exsorption of etoposide in the small intestine an *in situ* single-pass perfusion study was performed.[13] Male Sprague-Dawley rats were given an i.p. injection of urethane (1.5 g/kg body weight). Both ends of the small intestinal segment were cannulated with Teflon tubing. The segment was perfused using a syringe pump with culture medium at a flow rate of 0.25 ml/min. The abdomen was covered with saline-

soaked gauze to maintain moisture. The jugular vein, cannulated with heparinized tubing (0.02 in ID and 0.037 in OD), was infused with etoposide (200 μg/h) and inhibitor at a flow rate of 1.0 ml/h. Blood samples were drawn via the carotid artery. Both blood and perfusate samples were collected hourly. Total body clearance (CL_t) was determined by dividing the rate of infusion by the average plasma concentration of 5–8 h. The intestinal exsorption clearance (CL_{exs}) was determined by dividing the rate of intestinal luminal excretion by plasma drug concentration at the same period.

Intestinal absorption of etoposide *in vivo*

The absorption of etoposide was measured by an *in situ* circulated perfusion study similar to that mentioned in the exsorption study. Both ends of tubing were connected to a peristaltic pump and were circulated with 100 ml Tyrode solution containing 400 μg/ml etoposide at a flow rate of 18–20 ml/h. A volume of 0.5 ml circulated solution was taken hourly and blood samples were drawn via jugular vein hourly.

HPLC assay

Etoposide was analyzed by a modified HPLC method.[4] Samples were extracted by dichloromethane and a volume of 100 μl was injected into the HPLC system. The HPLC system consisted of a pump (600E; Waters, Milford, MA) at a flow rate of 1.5 ml/min; an automatic injector (WISP 710; Waters), a reverse phase column (μBondapack-C18; Waters), a fluorescence detector (1046A; Hewlett Packard, Avondale, PA) with λ_{ex} at 215 nm and λ_{em} at 328 nm, and an integrator (3395A; Hewlett Packard). The mobile phase contained methanol:water:glacial acetic acid = 43:57:0.1.

Results

Everted sac absorption study

When 100 μg/ml etoposide was used in the jejunal everted sacs, approximately 1.45 and 20.36 μg/ml of drug was detected after 10 and 90 min, respectively. With the addition of either 1 mg/ml of quinidine sulfate or 500 μM hydroxyzine, the transport of etoposide to the serosal site significantly increased 2- to 4-fold ($p < 0.05$) (Figure 2). After 90 min, the concentrations of etoposide in the jejunal serosal site were 80.34 and 41.37 μg/ml in the presence of quinidine and hydroxyzine, respectively. A similar trend was observed when ileal sacs were chosen. The concentrations of etoposide inside the ileal sacs were

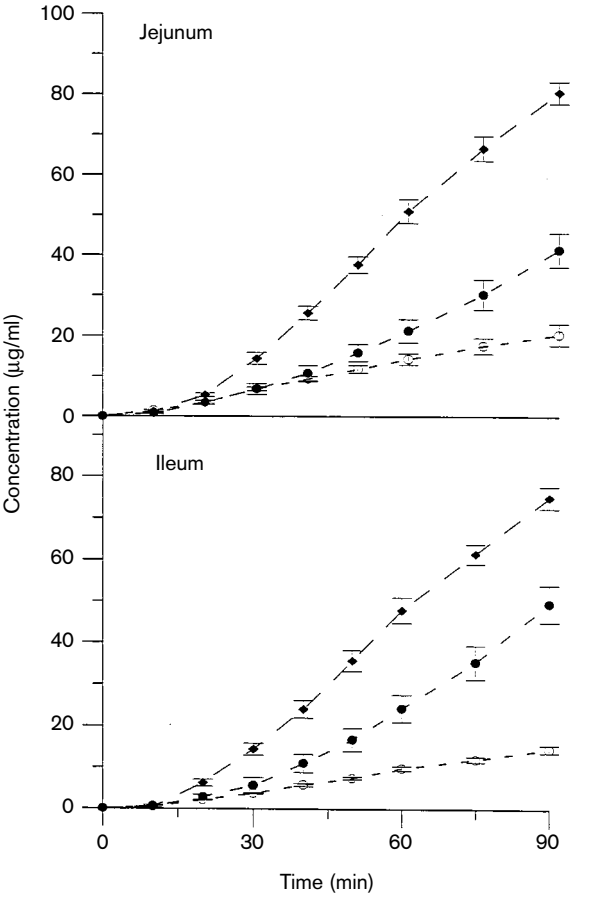

Figure 2. Effects of quinidine and hydroxyzine on the absorption of etoposide in rat intestinal everted sacs. Each point is the mean ± SEM of six experiments: (○) control, (◆) 1 mg/ml quinidine and (●) 500 μM hydroxyzine.

14.48 and 49.41 μg/ml in the absence or presence of hydroxyzine after 90 min (Figure 2).

Everted sac exsorption study

The amount of etoposide appearing outside the sacs increased gradually. A concentration of 3.17 ± 0.13 and 3.29 ± 0.19 μg/ml of etoposide was detected after 90 min in the jejunum and ileum, respectively (Figure 3). The presence of hydroxyzine could significantly reduce the efflux and approximately 2.4 μg/ml of etoposide was detected after 90 min.

Everted sac exsorption study of RH123

When RH123 was chosen as a substrate in the exsorption study, 0.21 ± 0.04 μg/ml of RH123 was measured after 90 min in the jejunum, while concentrations of 0.15 ± 0.03 and 0.14 ± 0.03 μg/ml were ob-

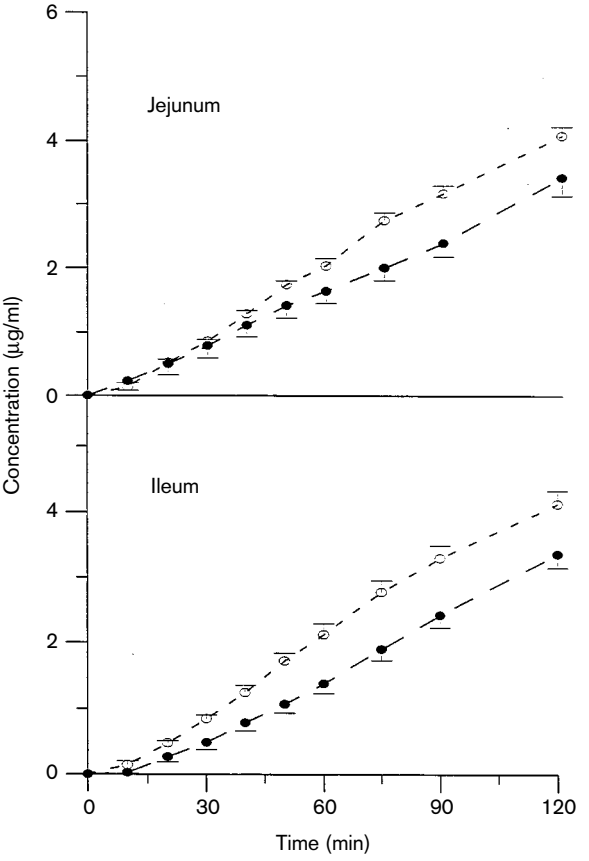

Figure 3. Effect of hydroxyzine on the exsorption of etoposide in rat intestinal everted sacs. Each point represents the mean ± SEM of six experiments: (○) control and (●) 500 μM hydroxyzine.

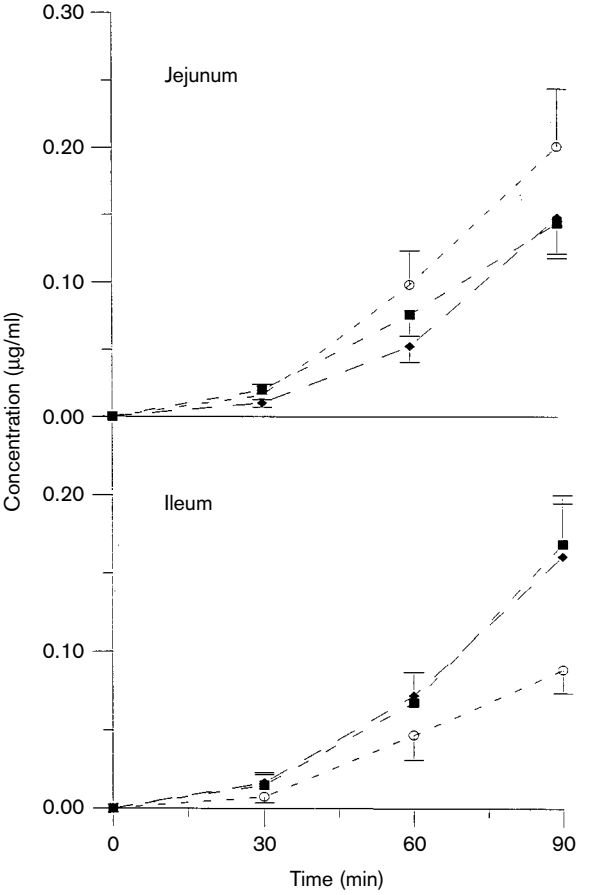

Figure 4. Effects of quinidine and hydroxyzine on the exsorption of RH123 in rat intestinal everted sacs. Values are the means ± SEM of six experiments: (○) control, (◆) 1 mg/ml quinidine and (■) 500 μM hydroxyzine.

served in the presence of quinidine and hydroxyzine, respectively (Figure 4). However, in the ileum, the addition of either quinidine or hydroxyzine significantly enhanced the efflux of RH123 to the lumen.

In vivo exsorption study

When the jugular vein was infused with 200 μg/h etoposide, the plasma concentration increased rapidly in the initial 2 h, then remained at a concentration of 0.9 μg/ml. With the addition of either 1 mg/ml quinidine or 500 μM hydroxyzine, the plasma concentration almost doubled at 8 h ($p < 0.0001$) (Figure 5). In the meantime, the appearance of etoposide in the intestinal lumen was approximately 1.86 μg/ml after 8 h. There was no significant difference when either quinidine or hydroxyzine was added (Figure 5). The intestinal clearance was 110.94 ± 21.24 ml/h/kg in the control group; reduced clearance was observed in the presence of either quinidine or hydroxyzine ($p < 0.05$). The total body clearance of etoposide was also significantly decreased by quinidine ($p < 0.05$). The total body clearances of etoposide were 826.70 ± 135.85 ml/h/kg in the control group, and 330.37 ± 70.46 or 443.99 ± 39.04 ml/h/kg when co-infused with quinidine and with hydroxyzine, respectively (Table 1).

Table 1. Effect of hydroxyzine on the clearance of etoposide in rats

	CL_{int} (ml/h/kg)	CL_t (ml/h/kg)
Control	110.94 ± 21.24	826.70 ± 135.85
+ Quinidine	53.56 ± 4.60[a]	330.37 ± 70.46[a]
+ Hydroxyzine	57.63 ± 8.34[a]	443.99 ± 39.04[a]

[a] $p < 0.05$ between the control and experimental group.

In vivo absorption study

When the concentration of 400 μg/ml etoposide was administered intraluminally at a rate of 20 ml/h,

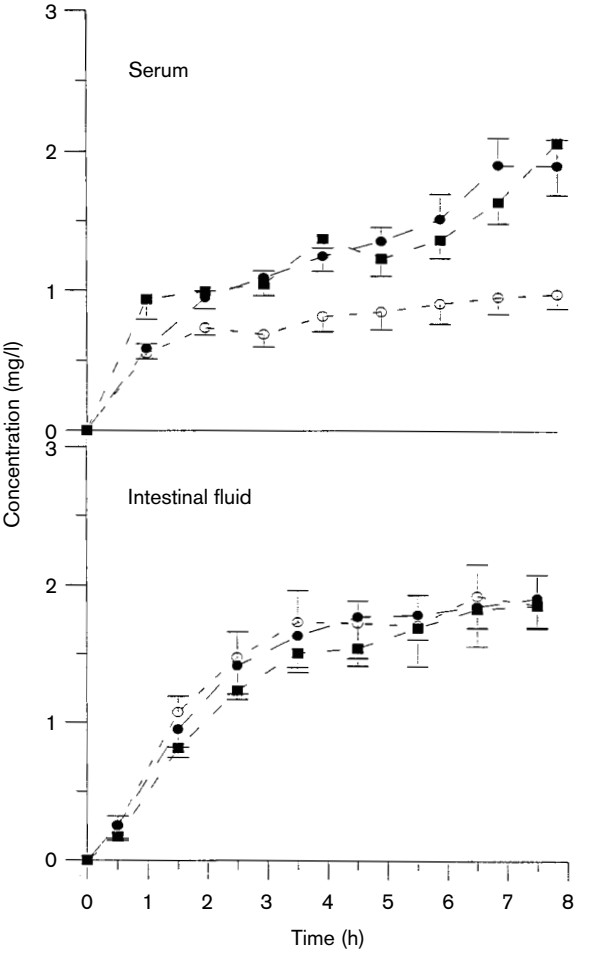

Figure 5. The concentration–time profile of etoposide in the exsorption study. (Upper) Concentration in plasma samples: (○) control, (●) 1 mg/ml quinidine. (■) 500 μM hydroxyzine. (Lower) Concentration in the intestinal fluid. Data are expressed as the mean ± SEM of six experiments.

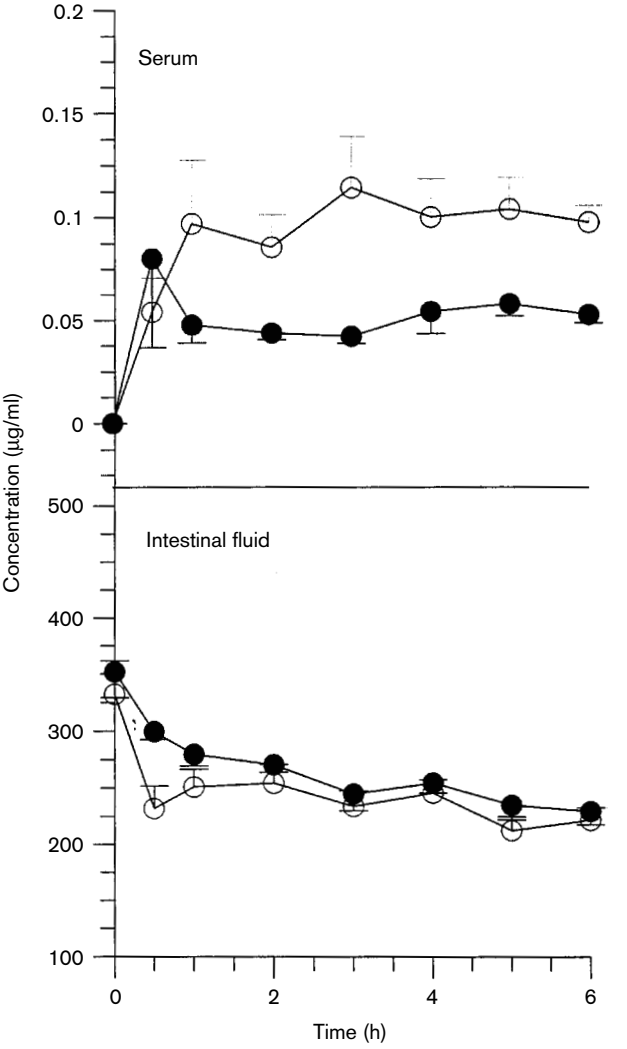

Figure 6. The concentration–time profile of etoposide administered intraluminally. (Upper) Concentration in plasma samples: (○) control and (●) 500 μM hydroxyzine. (Lower) Concentration in the intestinal fluid. Data represent means ± SEM of four experiments.

plasma etoposide concentration increased rapidly over the first 1 h and reached a plateau concentration of approximately 0.1 μg/ml (Figure 6). However, the presence of 500 μM hydroxyzine could significantly decrease the steady-state etoposide concentration 2-fold, where the steady-state concentration reached about 0.055 μg/ml (Figure 6). The etoposide concentration in the lumen was constant throughout the study period.

Discussion

A case report in 1992 mentioned that severe adverse effects were observed in a breast cancer patient who received doxorubicin and terfenadine.[9] Further study proved that terfenadine is an inhibitor of P-gp.[10] Therefore, an antihistamine with a chemical structure similar to terfenadine was chosen to assess its feasibility as a P-gp inhibitor. The current study of *in vitro* absorption demonstrated that hydroxyzine significantly enhanced the transport of etoposide 2-fold from lumen to serosa. This indicated that hydroxyzine could reduce the efflux and/or increase the absorption of etoposide in the small intestine. The data from the exsorption study further showed that hydroxyzine could reduce the efflux of etoposide by 25% after 90 min. RH123 is a P-gp substrate in various tissues.[14] Thus it was chosen to further evaluate the role of P-gp in the interaction between hydroxyzine and etoposide, with results suggesting that hydroxyzine might be a P-

gp inhibitor. It has been shown that etoposide is also a substrate of multidrug resistance-associated protein (MRP) which is expressed in the small intestine.[15,16] Whether MRP participated in the drug interaction requires further studies.

In rat jejunum, hydroxyzine might inhibit P-gp to reduce the efflux of RH123 and in fact a lower concentration of RH123 in the jejunal lumen was observed. Reduction in RH123 concentration by either hydroxyzine or quinidine was to the same extent, but different from that using etoposide as a substrate. This suggested that different P-gp isoforms might be involved in the transport of RH123 and etoposide since there are two P-gp isoforms in rats. The study by Shapiro and Ling proposed at least two distinct drug-binding sites of P-gp.[17] The other possibility is that etoposide and RH123 bind to different sites of P-gp. In contrast, RH123 secretion was increased in the ileum when hydroxyzine was added. One explanation may be that some other transporter is involved for which hydroxyzine could also serve as an inhibitor for RH123 absorption in the ileum. Thus, an elevated RH123 concentration could be observed in the ileum.

The intestinal excretion was about 14% of the total clearance based on the results from the *in vivo* study. Both the intestinal and total clearances (CL_{int}, CL_t) were reduced by 50% in the presence of hydroxyzine. This suggests that interaction occurred not only in the small intestine, but also in some other tissue(s). Studies have already shown that P-gp is involved in etoposide resistance in other tissues such as lung, kidney and brain.[18–20] Therefore, hydroxyzine may also interact with etoposide at other tissues to a similar extent.

When etoposide was administered by i.v. infusion, a significant increase in serum etoposide concentration was observed. Since hydroxyzine interacts with etoposide to reduce its efflux, a lower etoposide concentration in the intestinal fluid was expected when etoposide was co-infused with hydroxyzine. However, no marked difference was observed in the intestinal fluid. This supported the hypothesis that some other transporter might be involved in reducing the efflux of etoposide to the intestinal lumen. A study by Asperen and his colleagues has demonstrated the difference in the disposition of P-gp substrate in mice.[21] Thus, this also suggests that the major site of interaction occurs at a place other than the small intestine and that the inhibition of bile secretion might play an important role. On the contrary, a significant reduction in etoposide plasma concentration was observed when etoposide was administered intraluminally in a higher dose, creating the possibility of activation of an efflux transporter. A detailed study is ongoing to try to clarify this issue.

In the *in vitro* study, there is a significant difference in the absorption of etoposide in the presence of quinidine or of hydroxyzine in either jejunum or ileum. However, the effects of these two compounds on the increase in plasma etoposide concentration *in vivo* were similar. This indicates that drug interaction occurs not only in the small intestine. It has been demonstrated that both quinidine and etoposide are substrates of cytochrome P450 3A4[22] and hydroxyzine is a CYP2D6 inhibitor.[23] This suggests that factors other than CYP3A may be involved in the interaction between etoposide and hydroxyzine.

Quinidine is a potent P-gp inhibitor.[24,25] However, the cardiac toxicity of quinidine renders this drug as a multidrug resistance inhibitor for clinical use. Other potent P-gp inhibitors such as cyclosporine A and verapamil exhibit a similar problem.[8] Hydroxyzine is an antihistamine with a wide therapeutic range. Quinidine and hydroxyzine exhibit equivalent effects on etoposide plasma concentration when given i.v. This provides valuable information for clinical applications. The current study showed that the effect of hydroxyzine on etoposide pharmacokinetics depends upon the route of administration. When etoposide is administered i.v. with hydroxyzine, the etoposide concentration should be monitored carefully to prevent toxicity. However, dosage adjustment is required to achieve efficacy when etoposide is administered orally with hydroxyzine. Further study will evaluate the effect of hydroxyzine on the tissue distribution of etoposide. In conclusion, this is a novel study to illustrate the interaction between etoposide and hydroxyzine for drug-resistant therapy.

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