

Effects of Naringin on the Bioavailability of Etoposide in Rats

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ABSTRACT – This study investigated the effect of naringin, a flavonoid, on the bioavailability of etoposide administered orally to rats. Etoposide (6 mg/kg) was administered orally to rats alone or with naringin (1, 4 or 12 mg/kg). Compared with the control group, the co-administration of etoposide with 4 and 12 mg/kg of naringin significantly ($p < 0.05$) increased the area under the plasma concentration-time curve (AUC) and the peak plasma concentration (C_{max}) of the oral etoposide. Consequently, the absolute bioavailability (AB) of etoposide in the presence (4 and 12 mg/kg) of naringin was significantly ($p < 0.05$) increased by 9.4–10.6% compared with the control group (7.4%). The relative bioavailability (RB) of etoposide was increased 1.13- to 1.44-fold compared to the control group. Enhanced bioavailability of etoposide might be due to inhibition of both cytochrome P450 (CYP) 3A4 in the intestine or liver and P-glycoprotein (P-gp) transport efflux of etoposide in the intestinal membrane. This data indicate that careful consideration of the dosage for therapy with etoposide is required in a case of clinical application of the co-administration of etoposide and naringin.

Key words – Etoposide, Naringin, Bioavailability, CYP3A4, P-gp, Rats

Etoposide is a semisynthetic derivative of podophyllotoxin, a naturally occurring compound extracted from the roots and rhizomes of the plants *Podophyllum peltatum* or *Podophyllum emodi*.^{1,2)} Etoposide is used in the treatment for a wide range of malignancies (e.g., small cell lung cancer, acute leukemia, lymphoma, testicular cancer).²⁾ Its mechanism of action is believed to be the inhibition of topoisomerase II enzyme and/or induction of direct DNA breaks.³⁾ Metabolism of etoposide includes cytochrome P450 (CYP)-catalyzed *O*-demethylation in liver microsomes of rats⁴⁾ and humans.⁵⁾ The main cytochrome P450 isoenzyme involved in the metabolism of etoposide is CYP3A4.⁶⁾ Etoposide was also reported to be a substrate for P-glycoprotein (P-gp) in inverted gut sacs prepared from rat jejunum and ileum.⁷⁾ Cyclosporine, a CYP3A4 and P-gp inhibitors, increased the plasma concentration and decreased the plasma clearance of etoposide in rats.⁸⁾ The cyclosporine analogue, PSC833 also increase the plasma concentration of orally administered etoposide in rats⁹⁾ and quinidine enhanced the uptake of etoposide from rat *in situ* perfused intestinal loops,⁷⁾ these compounds are known as CYP3A4 and P-gp inhibitor. Since CYP3A4 and P-gp share a variety of substrates, their function could modulate the bioavailability of many drugs by affecting their presystemic metabolism.^{10,11)}

Among flavonoids, naringin (4',5,7-trihydroxy-flavanone-7-rhamnoglucoside) is the predominant component in grapefruit

juice and transformed into naringenin by the bacteria in human intestinal microflora.^{12,13)} Both naringin and naringenin reduced apical efflux of vinblastine, a substrate of P-gp.¹⁴⁾ In addition, naringin and naringenin also inhibit CYP3A4-mediated metabolism and modulate P-gp transport in Caco-2 cell.¹⁵⁾ Therefore, naringin might act as a dual inhibitors of CYP 3A4 and P-gp. Several studies have demonstrated that grapefruit juice could alter the pharmacokinetics of certain drugs, such as felodipine, nifedipine and verapamil and suggested that the interaction of drugs with grapefruit juice may be caused by a combined inhibition of presystemic metabolism and P-gp during the intestinal drug absorption.¹⁶⁻¹⁸⁾ However, the components in grapefruit juice responsible for such an interaction have not been fully determined, although particular interest has been focused on the grapefruit flavonoid, naringin, furanocoumarin and 6',7'-dihydroxybergamottin.¹⁹⁻²¹⁾

It is possible that naringin as a dietary components with many health beneficial activity would be combined with anti-neoplastic agents in the anticancer therapy. Orally administration of naringin, as the CYP3A4 and P-gp inhibitor, might improve the erratic oral bioavailability of etoposide. Therefore, the present study investigated the effect of naringin on the bioavailability or pharmacokinetics of oral etoposide in rats.

Materials and Methods

Materials

Etoposide, naringin, podophyllotoxin [an internal standard

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of high performance liquid chromatographic (HPLC) analysis of etoposide] were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Etoposide injectable solution was purchased from Boryung chemical Co. (Seoul, Republic of Korea). Methanol and tert-butylmethylether were acquired from the Merck Co. (Darmstadt, Germany). Other chemicals were of reagent grade or HPLC grade.

A high performance liquid chromatograph (HPLC, Waters 1515 isocratic HPLC Pump, Waters 717 plus autosampler, WatersTM 474 scanning fluorescence detector, Waters Co., Milford, MA, USA), a microcentrifuge (National Labnet Co., NY, USA), a sonicator (Daihan Co., Republic of Korea), a HPLC column temperature controller (Phenomenex Inc., CA, USA) and a vortex-mixer (Scientific Industries Co., NY, USA) were used in this study.

Animal experiments and drug administration

Male Sprague-Dawley rats weighing (270 to 300 g) were purchased from the Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea), and were given access to a normal standard chow diet (No. 322-7-1) purchased from the Superfeed Co. (Wonju, Republic of Korea) and tap water *ad libitum*. Throughout the experiment, the animals were housed, four or five per cage, in laminar flow cages maintained at $22 \pm 2^\circ\text{C}$, and 50~60% relative humidity, under a 12:12 h light-dark cycle throughout the experiment. The animals were acclimatized in this environment for at least one week before the experimentation. The experiments were carried out in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the design for this study. The rats were randomly divided into four groups containing four animals in each group: (1) an oral control group, administered 6 mg/kg etoposide orally; (2) the oral combination groups, administered naringin (1, 4 or 12 mg/kg) 30 min prior to the oral etoposide; (3) an i.v. control group, intravenous administration 2 mg/kg of etoposide. The rats were fasted for at least 24 h prior to beginning the experiments. Each animal was anesthetized with ether and the right femoral vein (for drug administration for intravenous) and femoral artery (for blood sampling) were cannulated with polyethylene tube (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. Ltd., Tokyo, Japan).

The etoposide solution for oral administration was prepared by diluting the 20 mg/kg of etoposide injectable solution in distilled water and the solution for intravenous administration was diluted with 0.9% NaCl-injectable. Etoposide solution for

intravenous administration was injected through the femoral vein within 40 sec. The naringin was prepared by dissolving in distilled water. Blood samples (0.5 mL) were collected into heparinized tubes via the femoral artery at 0 (to serve as a control), 0.017 (at the end injection), 0.1, 0.25, 0.5, 0.75, 1, 2, 4, 6 and 10 h after the oral or i.v. administration of the etoposide solution. The blood samples were centrifuged at 13,000 rpm for 5 min and the plasmas stored at -40°C until HPLC analysis.

HPLC analysis

The plasma concentrations of etoposide were determined by a HPLC assay and a modification of the method reported by Liliemark *et al.*²²⁾ and Manouilov *et al.*²³⁾ Briefly, 50 μL of 50 ng/mL podophyllotoxin and 1.2 mL of tert-butylmethylether were mixed with 0.2 mL of the plasma sample in a 2.0 mL polypropylene microtube. The resulting mixture was vortex-mixed vigorously for 1 min and centrifuged at 13,000 rpm for 10 min. One mL of the upper layer was transferred to another microtube, evaporated under nitrogen at 38°C . The residue was dissolved with 0.2 mL of 50% methanol and centrifuged (13,000, 5 min). A total of 50 μL of the supernatant was injected to the HPLC system.

The fluorescence detector was operated at an excitation wavelength of 230 nm with an emission cut-off filter of 330 nm. A Symmetry[®] C₁₈ column (4.6 \times 150 mm, 5 μm , Waters Co., Milford, MA, USA) was used at a temperature of 30°C that was set by HPLC column temperature controller. The mobile phase consisted of methanol:water:acetic acid (50:50:0.5, v/v/v). The flow rate was maintained at 1.0 mL/min. The retention times of etoposide and an internal standard were approximately 5.4 and 11.1 min, respectively. The calibration curve of etoposide was linear within the range of 10~500 ng/mL. The detection limit of etoposide in plasma was 10 ng/mL. The intra- and inter-day variation coefficients of etoposide in plasma sample were below 13.6%.

Pharmacokinetic analysis

The plasma concentration data were analyzed by noncompartmental method using WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of etoposide concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of etoposide in plasma were obtained by visual inspection of the data from the concentration-time curve. The area under the plasma concentration-time curve

Table I—Mean (\pm S.D.) Pharmacokinetic Parameters of Etoposide after Oral (6 mg/kg) and Intravenous (2 mg/kg) Administration of Etoposide to Rat in the Presence or Absence of Naringin

Parameters	Control	Etoposide + Naringin			IV
		1 mg/kg	4 mg/kg	12 mg/kg	
AUC (ng/mL·h)	605 \pm 148	681 \pm 161	772 \pm 183*	871 \pm 209**	2742 \pm 570
C _{max} (ng/mL)	276 \pm 65	304 \pm 83	339 \pm 82*	368 \pm 91**	
T _{max} (h)	0.5	0.5	0.5	0.5	
t _{1/2} (h)	2.0 \pm 0.49	2.2 \pm 0.61	2.4 \pm 0.62	2.6 \pm 0.69	1.8 \pm 0.38
AB (%)	7.4 \pm 2.0	8.3 \pm 2.3	9.4 \pm 2.5*	10.6 \pm 2.8**	
RB (%)	100	113	128	144	

Mean \pm S.D. (n=6), *p<0.05, **p<0.01 compared to control.

AUC: area under the plasma concentration–time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach peak concentration; t_{1/2}: terminal half-life; AB (%): absolute bioavailability; RB (%): relative bioavailability.

(AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite (AUC_{0-∞}) was obtained by the summation of AUC_{0-t} to the extrapolated area determined by C_{last}/K_{el}. Total body clearance was calculated by Dose/AUC. The absolute bioavailability (AB%) of etoposide was calculated by AUC_{oral}/AUC_{i.v.} × Dose_{i.v.}/Dose_{oral} × 100, and the relative bioavailability (RB%) of etoposide was estimated by AUC_{combine}/AUC_{control} × 100.

Statistical analysis

All mean values are presented with their standard deviation (Mean \pm S.D.). The pharmacokinetic parameters were compared using a one-way ANOVA, followed by *a posteriori* testing with the use of the Dunnett's correction. Differences were considered to be significant at a level of *p*<0.05.

Results and Discussion

The plasma concentration-time profiles of etoposide after oral administration of etoposide (6 mg/kg) in the presence or absence of naringin²⁴ (1, 4 or 12 mg/kg) are illustrated in Fig. 1, and the pharmacokinetic parameters for etoposide are shown in Table I. As shown in Table I, the presence (4 or 12 mg/kg) of naringin significantly altered the pharmacokinetic parameters of the oral etoposide. Compared to the oral control group (given 6 mg/kg of etoposide alone), the presence of naringin significantly (4 mg/kg, *p*<0.05; 12 mg/kg, *p*<0.01) increased the area under the plasma concentration-time curve (AUC) and the peak plasma concentration (C_{max}) of the oral etoposide. The absolute bioavailability (AB%) of etoposide was significantly (4 mg/kg, *p*<0.05; 12 mg/kg, *p*<0.01) increased by 9.4~10.3% compared to the oral control group (7.4%). The relative bioavailability (RB%) of the oral etoposide was increased

1.13- to 1.44-fold. These results suggest that presence of naringin might inhibit the CYP3A4 and P-gp of oral etoposide. Because, etoposide is a substrate of P-gp and mainly metabolized by CYP3A4 in the intestine and/or liver.^{6,7} P-gp exists with CYP3A4 in the apical membrane of intestine,^{25,26} and both of them act synergistically in regulating the bioavailability of many orally ingested anticancer agents. Naringin and naringenin also inhibit CYP3A4-mediated metabolism and also modulate P-gp transport in Caco-2 cell.¹⁵ As shown in Table I, the presence of 4 or 12 mg/kg of naringin significantly increased AUC and C_{max} of the etoposide. This result is coincident with the previous study in that the presence of naringin significantly increased AUC and C_{max} of paclitaxel, a P-gp and CYP3A4 substrate,²⁷ and the presence of morin, a flavonoid, significantly increased AUC and C_{max} of etoposide by inhi-

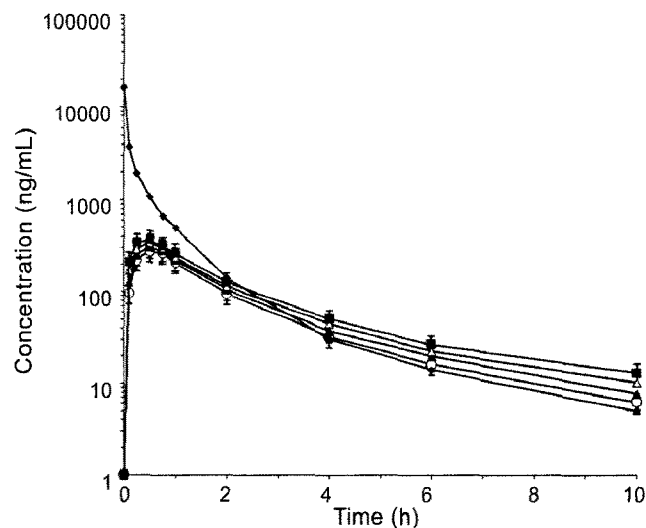


Figure 1—Mean arterial plasma concentration-time profiles of etoposide after intravenous (\blacklozenge ; n=6) or oral administration of etoposide at a dose of 6 mg/kg in the presence of naringin at doses of 1 (\blacktriangle ; n=6), 4 (\triangle ; n=6) and 12 mg/kg (\blacksquare ; n=6) or absence (\circ ; n=6) of naringin to rats. Bars represent standard deviation.

bition of the P-gp and CYP3A4 in intestine and/or liver in rats.²⁸⁾ The presence of naringin has not given the significant change of the terminal half-life ($t_{1/2}$), the time to reach peak plasma concentration (T_{max}) and peak plasma concentration (C_{max}) of etoposide.

Conclusion

Enhanced bioavailability of etoposide might be due to inhibition of both cytochrome P450 (CYP) 3A4 in the intestine or liver and P-glycoprotein (P-gp) transport efflux of etoposide in the intestinal membrane. Dosage adjustment of etoposide should be taken into consideration for safe therapy in clinical practice of etoposide with naringin, if this result will be confirmed in clinical study.

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