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Enhanced bioavailability of paclitaxel after oral coadministration with flavone in rats

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Abstract

The purpose of this study was to investigate the effect of flavone on the bioavailability of paclitaxel orally coadministered in rats. Paclitaxel (40 mg/kg) and flavone (2, 10, 20 mg/kg) were orally administered to rats orally. The plasma concentration of paclitaxel with flavone increased significantly ($P < 0.01$) compared to that of paclitaxel control. Area under the plasma concentration–time curve (AUC) of paclitaxel with the dose of 2–20 mg/kg flavone was significantly ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) higher than that of control. AUCs of paclitaxel were increased dose-dependently in the dose range of flavone. The absorption rate constant (K_a) of paclitaxel with the dose of 10–20 mg/kg flavone was significantly increased ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to that of control. Peak concentration (C_{max}) of paclitaxel with the dose of 10–20 mg/kg flavone were significantly increased ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to that of control. Half-life ($t_{1/2}$) of paclitaxel with the dose of 10–20 mg/kg flavone was significantly prolonged ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to that of control. The relative bioavailability increased significantly by about 2.38- or 3.10-fold ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to that of paclitaxel control. Based on these results, It might be considered that the bioavailability of paclitaxel coadministered with flavone was significantly enhanced by the both inhibition of cytochrome P-450 and the P-gp efflux pump in the intestinal mucosa. It could be possible to administer paclitaxel orally besides the established IV route.

Keywords: Paclitaxel; Flavone; Pharmacokinetics; Bioavailability

1. Introduction

Paclitaxel (Taxol®) is an antineoplastic agent that is derived from the bark of the Pacific yew tree (*Taxus brevifolia*) (Wani et al., 1971). In contrast to vinca alkaloids, the anticancer action of taxol is that it inhibits cellular growth by promoting and stabilizing microtubule assembly by noncovalent interaction with tubulin, thereby blocking cell replication in the late G_2 mitotic phase of the cell cycle (Kumar, 1981; Manfredi and Horwitz, 1984). Because of its poor water solubility, paclitaxel is currently formulated in taxol and a mixture of polyoxyethyleneglycerol triricinolate 35 (Cremophor EL) and dehydrated ethanol (1:1, v/v) for IV dosage form. Cremophor EL, itself, is toxic and produces vasodilatation, labored breathing, lethargy and hypotension when administered intravenously. One mediator of hypersensitivity reactions is endogenous histamine release, and prophylaxis to counteract histaminergic mechanisms reduces

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the incidence of hypersensitivity reactions. It has been reported that the human toxicity of paclitaxel included myelosuppression, emesis, weight loss, hepatic dysfunction and increases in total plasma lipids, cholesterol and triglyceride and so on (Rowinsky et al., 1993). Paclitaxel has been used to treat ovarian carcinoma, breast carcinoma, leukemia, melanoma, prostate carcinoma and other cancers, and has become especially important for the management of ovarian and breast carcinoma (Rowinsky et al., 1990; McGuire et al., 1989; Sarosy et al., 1992; Holmes et al., 1991).

Paclitaxel has a very large volume of distribution in the body, and is highly bound by plasma protein, primarily albumin (95–98%) (Wiernik and Paietta et al., 1987). Its disposition by the liver and bile is higher than by other tissue (Hiroshi et al., 1994). Less than 6–10% of administered paclitaxel was recovered as unchanged drug in the urine of treated patients (Wiernik and Paietta et al., 1987; Wiernik and Einzig et al., 1987; Brown et al., 1991). Paclitaxel is mainly metabolized through the liver and undergoes biliary excretion (Cresteil et al., 1994; Kumar et al., 1994; Rahman et al., 1994; Sonnichsen et al., 1995). In humans, the total fecal excretion is approximately 70% of paclitaxel dose, with 6α-hydroxypaclitaxel being the major metabolite (Walle et al., 1995). Paclitaxel, however, is very poorly absorbed when administered orally. This poor bioavailability would result from cytocrome p-450 (CYP 3A) and *p*-glycoprotein (P-gp) efflux pump, multidrug transporter, which is present abundantly in the gastrointestine (Cresteil et al., 1994; Kumar et al., 1994; Rahman et al., 1994; Sonnichsen et al., 1995; Sparreboom et al., 1997).

In an attempt to develop safe formulations, many studies have been directed to new oral formulations. It has been speculated that, in some cases, the poor absorption of drugs after oral administration results from the activity of a multidrug transporter, a membrane-bound P-gp, which functions as an energy-dependent transporter or efflux pump to decrease intracellular accumulation of drugs by extruding xenobiotics from the cell (Sparreboom et al., 1997).

Flavonoids are regarded as a new class of chemosensitizers, which interact with both cytosolic domains of P-gp and its ATP binding site (Conseil et al., 1998). They also have been reported as various inhibitors of CYP enzymes or antioxidant agents (Peter et al., 2002).

Flavone (2-phenyl-4*H*-1-benzopyran-4-one) as a member of the flavonoid class, occurring in many cereal grains as well as in dill weed (Middleton and Kandaswami, 1993), as topoisomerase I inhibitor is usually applied as a second-line pharmacotherapeutic in advanced colorectal cancers to promote apoptosis (Cunningham and Glimelius, 1999; Whitacre et al., 1999). Flavone was found to reduce cell proliferation in HT-29 human colon cancer cells in a concentration-dependent manner, and was found to be as effective as the classical antitumor agent camptothecin. It potently induces differentiation as well as apoptosis. Quercetin, naringin, GF120918 and cyclosporin have increased bioavailability of some drugs by inhibit of CYP3A and P-pg (Choi et al., 2004; Scambia et al., 1995; Bardelmeijer et al., 2000; Malingre et al., 2001). But there is no investigation on flavone to evaluate if it potentiates the ability of inhibition of P-gp and CYP enzymes or not. The purpose of this study was to investigate the bioavailability of paclitaxel after oral administration of paclitaxel alone and also paclitaxel coadministered with flavone.

2. Materials and methods

2.1. Materials

Paclitaxel was purchased from Brystol-Myers Squibb Co. (NY, USA). Flavone, *n*-butyl *p*-hydroxybenzoate (butylparaben) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, methanol, ether and ammonium acetate were HPLC grade from Merck Co. (Darmstadt, Germany). The other chemicals were reagent grade and used without further purification. Centrifugal evaporator (Rikakikai Co., Japan), microcentrifuge (National Labnet, USA), sonicatior (Daihan Co., Korea), refrigerated bath circulator, and rotamix (Seoulin Bioscience, Korea) were used.

2.2. Animal experiments and drug administration

Male Sprague–Dawley rats (270–300 g) were purchased from Daehan Laboratory Animal Research and Co. (Choongbuk, Korea), and had free access to normal standard chow diet (Jae II Chow, Korea) and tap water. Throughout the experiment, the animals were

housed, four or five per cage, in laminar flow cages maintained at 22±2 °C, 50–60% relative humidity, under a 12-h light–dark cycle. The animals were kept in these facilities for at least one week before the experiment. This experiment was 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 in our institution (Chosun University) approved the present study.

Sprague–Dawley rats were fasted for at least 24 h prior to experiments and were given water freely. Each rat was anaesthetized with ether. The right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, USA) for blood sampling. In control group, paclitaxel suspension was prepared by adding paclitaxel (40 mg/kg) to distilled water (1.0 ml) containing tween80 (10 μl) and stirring for 1 h. The paclitaxel suspension was administered orally to rats. In coadministered group, flavone (2, 10, 20 mg/kg) suspensions were prepared in distilled water (0.6 ml) containing tween80 (10 μl) and stirring for 1 h. The flavone suspensions were administered orally to rats. Twenty minutes after flavone suspension administration, the paclitaxel suspension was administered to rats orally. In IV group, pacltaxel (2 mg/kg solved in 0.3 ml saline) was administered into femoral vein. Blood samples (0.6 ml) were withdrawn from the femoral artery at 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h, after administration of the drug. The plasma samples were centrifuged at 5000 rpm for 5 min. The plasmas were stored at −40 °C until the HPLC analysis.

2.3. HPLC assay

Plasma concentrations of paclitaxel were determined by HPLC (high performance liquid chromatography) assay by modifications of the methods of Catalin and Mase (Catalin et al., 1998; Mase et al., 1994). Briefly, a 0.1-ml of *n*-butyl *p*-hydroxybenzoate (2 μg/ml) as the internal standard and a 0.2-ml of 0.2 M ammonium acetate buffer (pH 5.0) and 4 ml of ether were added to a 0.25-ml of plasma samples. It was then mixed on the rotamix for 15 min and centrifuged at 3000 rpm for 10 min, a 3.6-ml of organic layer was transferred to a clean test tube and evaporated under centrifugal evaporator at 30 °C. The residue was then dissolved in 0.3 ml of mobile phase (ACN:MeOH:2 mM phosphate buffer (pH 5.0), 38:22:40) and centrifuged 13,000 rpm for 5 min and a 100 μl of the solution was injected into the HPLC system.

The HPLC system consisted of Waters 1515 isocratic HPLC Pump, Waters 717 plus autosampler, Waters 2487 dual λ absorbance detector (Waters Co., Milford, MA, USA) and computing integrator. The detector wavelength was set at 227 nm and the column was at room temperature. The column used was a symmetry C_{18} column (4.6 mm × 150 mm, 5 μm, Waters Co., USA). Mixtures of acetonitrile:methanol:2 mM phosphate buffer (pH 5.0) (38:22:40 v/v/v) were used as the mobile phases at a flow rate of 1.2 ml/min. The mobile phase was filtered by passing through a 0.45-μm pore size membrane filter. The retention times were as follows: internal standard, 5.358 min and paclitaxel, 7.867 min (Fig. 1).

2.4. Pharmacokinetic analysis

Pharmacokinetic parameters in terms of a one compartment open model were calculated with a nonlinear least square regression using a MULTI program (Yamaoka et al., 1981). The parameter value was estimated by simplex method. The final value was estimated at the AIC (Akaike's information criterion) values. The area under the plasma concentration–time curves (AUC) was calculated by trapezoidal rule.

The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were determined by a visual inspection of the experimental data. The elimination rate constant (K_{el}) was calculated by regression analysis from the slope of the line, and the half-life ($t^{1/2}$) of the drug was obtained by $0.693/K_{el}$. The absolute bioavailability of paclitaxel after the oral administration compared to the IV administration was calculated as follows:

$$\text{Absolute bioavailability (AB)} = \frac{\text{Oral AUC}}{\text{IV AUC}} \times \frac{\text{IV dose}}{\text{Oral dose}} \times 100$$

The relative bioavailability of paclitaxel after oral administration was calculated as follows:

$$\text{Relative bioavilability} = \frac{\text{AUC combined}}{\text{AUC control}} \times 100$$

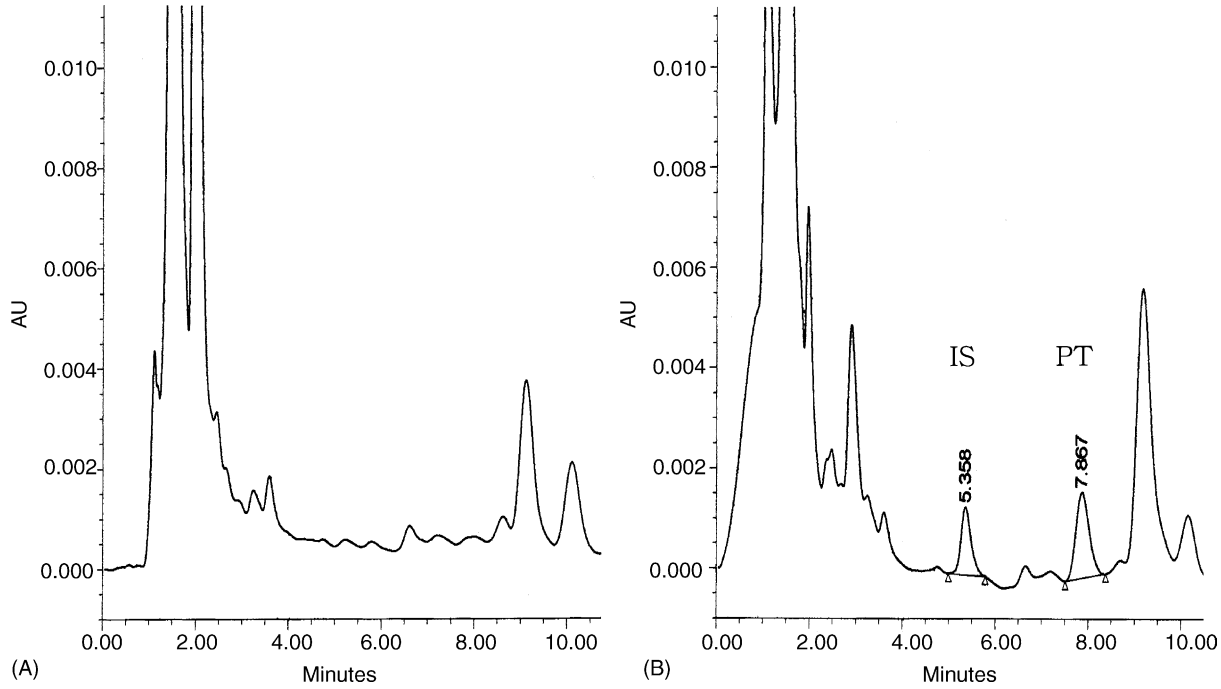

Fig. 1. Chromatograms of blank plasma (A) and plasma spiked (B) with internal standard (IS, 5.358 min) and paclitaxel (PT, 7.867 min).

2.5. Statistical analysis

All means were presented with their standard deviation (Mean ± S.D.). Unpaired Student's *t*-test was utilized to determine a significance difference between paclitaxel control and coadministered with flavone. Differences were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1. Pharmacokinetics of paclitaxel

The plasma concentrations of paclitaxel after oral administration of paclitaxel (40 mg/kg) control and paclitaxel coadministered with various doses of flavone (2, 10, 20 mg/kg) were shown in Fig. 2. The plasma concentrations of paclitaxel with flavone were increased significantly ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to that of paclitaxel control.

The bioavailability and pharmacokinetic parameters of paclitaxel after administration of paclitaxel or paclitaxel coadministered with flavone were shown in Table 1. Areas under the plasma concentration–time curve (AUC) of paclitaxel with flavone were significantly higher ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) than the control, except for 2 mg/kg. AUCs of paclitaxel were increased dose-dependently in the dose range of flavone. The absorption rate constant (K_a) of paclitaxel with flavone were significantly increased ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to that of control, except for 2 mg/kg. Peak concentration (C_{max}) of paclitaxel with flavone were significantly increased ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to those of control, except for 2 mg/kg. Time to reach peak concentration (T_{max}) of paclitaxel with flavone were significantly shortened ($P < 0.05$ at 20 mg/kg) compared to that of control. The terminal half-life ($t_{1/2}$) of paclitaxel with flavone were significantly prolonged ($P < 0.05$ at 10 mg/kg, $P < 0.01$ at 20 mg/kg) compared to that of control, except for 2 mg/kg. The absolute bioavailability (AB%) of the paclitaxel was increased significantly ($P < 0.01$) by flavone (range between 3.7–6.4%). The relative bioavailability (RB%) of paclitaxel

Table 1
Pharmacokinetic parameters of paclitaxel after oral coadministration of paclitaxel (40 mg/kg) with flavone in rats

Parameters	Paclitaxel control	Flavone dose			IV (2 mg/kg)
		2 mg/kg	10 mg/kg	20 mg/kg	
K_a (h^{-1})	2.65 ± 0.54	3.56 ± 0.68	4.11 ± 1.10*	4.32 ± 1369**	
AUC_{0-24} (ng/ml h)	1651 ± 407	2967 ± 713	3926 ± 932*	5123 ±	3990 ± 1012
C_{max} (ng/ml)	112 ± 28.8	148 ± 41.2	190 ± 50.3*	209 ± 52.2**	
T_{max} (h)	1.6 ± 0.61	1.2 ± 0.31	1.2 ± 0.42	1.1 ± 0.40*	
K_{el} (h^{-1})	0.074 ± 0.016	0.054 ± 0.011	0.052 ± 0.009*	0.043 ± 0.009**	0.080 ± 0.021
$t_{1/2}$ (h)	9.31 ± 1.81	12.72 ± 2.59	13.19 ± 3.34*	16.12 ± 4.08**	8.66 ± 2.22
AB (%)	2.1	3.7	4.9	6.4	100
RB (%)	100	179	238	310	

Mean ± S.D. ($n = 6$), $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control; K_a: absorption rate constant; AUC_{0-24} area under the plasma concentration–time curve from 0 to 24 h; C_{max}: peak concentration T_{max}: time to reach peak concentration; K_{el}: elimination rate constant; $T_{1/2}$: terminal half-life; AB (%): absolute bioavailability; RB (%): AUC rate compared to AUC control.

with flavone was 2.4-to 3.1-fold higher than the control.

These results were consistent with the result reported by Scambia et al. and Choi et al., in that flavonoid (quercetin and naringin) increased bioavailability of paclitaxel and adriamycin by inhibition of P-gp pump efflux or cytochrome P-450 (CYP 3A). This result was also consistent with the result reported by Bardelmeijer et al. and Malingre et al., in that GF 120918 and Cyclosporin increased bioavailability of paclitaxel and docetaxel, respectively.

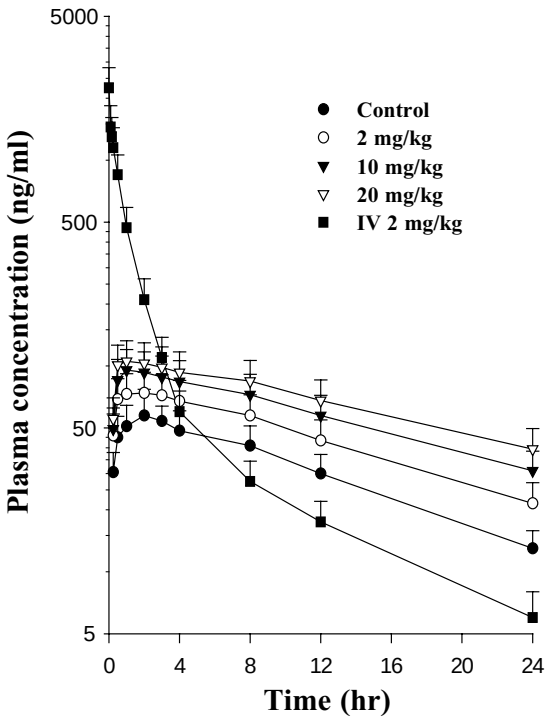

Fig. 2. Mean plasma concentration–time curves of paclitaxel after oral coadministration of paclitaxel (40 mg/kg) with flavone in rats.

Paclitaxel is reported to be metabolized by cytochrome P-450 (CYP 3A) in both the liver and epithelial cells of small intestine (Kumar et al., 1994; Rahman et al., 1994; Sonnichsen et al., 1995). In addition, the absorption of paclitaxel was inhibited by the P-pg efflux pump in the intestinal mucosa (Sparreboom et al., 1997). Flavone affected the bioavailability of paclitaxel similar to those of quercetin, anaringin and GF 120918.

Based on these results, It might be considered that the bioavailability of paclitaxel coadministered with flavone was significantly enhanced by the both inhibition of cytochrome P-450 and the P-gp efflux pump in the intestinal mucosa. It could be possible to administer paclitaxel orally besides the established IV route.

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