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Enhanced paclitaxel bioavailability after oral administration of pegylated paclitaxel prodrug for oral delivery in rats

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Abstract

The bioavailability and pharmacokinetic parameters of paclitaxel in a PEGylated paclitaxel prodrug were studied after the oral administration of paclitaxel (25, 50, 100 mg/kg) and prodrug (87.5, 175, 350 mg/kg) in rats. The area under the plasma concentration–time curve (AUC) of paclitaxel by oral paclitaxel were 836, 1602 and 3076 ng/ml h, which increased dose-dependently ($P < 0.006$, $r = 0.9996$). The AUCs of paclitaxel by the oral paclitaxel prodrug were 1646, 3079 and 5998 ng/ml h, also increased dose-dependently ($P < 0.003$, $r = 0.9999$). The AUC of paclitaxel by the intravenous administration of paclitaxel (2 mg/kg) was 3992 ng/ml h. The mean absolute bioavailability (AB%) of paclitaxel was 1.6% by the oral administration of paclitaxel. The mean AB% of paclitaxel by the prodrug was 6.3%, which was 3.94-fold higher than the oral paclitaxel. The peak concentration of paclitaxel (C_{max}) in the dose of 350 mg/kg (50 mg/kg as paclitaxel) of prodrug was 339 ng/ml, which was significantly higher ($P < 0.01$) than the dose of 50 mg/kg of paclitaxel (104 ng/ml). At the same dose of paclitaxel, the AUC of paclitaxel in the prodrug resulted in a remarkable increase, approximately four-fold compared to the oral paclitaxel. It might be considered that the significantly enhanced bioavailability of paclitaxel by the prodrug, which is water-soluble and easy to permeat through the intestinal mucosa, is due to the avoidance of being inhibited by *p*-glycoprotein efflux pump in the intestinal mucosa and reduction of metabolism by cytochrome-p-450 (CYP3A) in epitherial cells of small intestine. It appears that the development of oral paclitaxel preparations as a prodrug is possible, which will be more convenient than the IV dosage form.

Keywords: Paclitaxel; Bioavailability; Pharmacokinetics; PEGylated prodrug

1. Introduction

Paclitaxel (Taxol®) is a 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 to inhibit cellular growth by both promoting and stabilizing the microtubule assembly by a noncovalent interaction with tubulin, thereby blocking cell replication in the late G_2 mitotic phase of the cell cycle (Kumar, 1981; Manfedi and Horwitz, 1984). Because of its poor water solubility, paclitaxel is currently formulated in taxol and a mixture of polyoxyethyleneglycerol triricinoleate 35 (Cremophor EL) and dehydrated ethanol USP (1:1, v/v). Cremophor EL itself is toxic when administered intravenously and produces vasodilatations, labored breathing, lethargy and hypotension. One mediator of the hypersensitivity reactions is the endogenous histamine release, and a prophylaxis to counteract the histaminergic mechanisms reduces

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the incidence of the hypersensitivity reactions. It has been reported that the human toxicity of paclitaxel includes myelosuppression, emesis, weight loss, hepatic dysfunction and increases the total plasma lipids, cholesterol and triglyceride, etc. (Rowinsky et al., 1993). Paclitaxel has been used to treat ovarian carcinoma, breast carcinoma, leukemia, melanoma, prostate carcinoma etc, and has become particularly important for managing ovarian and breast carcinoma (McGuire et al., 1989; Rowinsky et al., 1990; Holmes et al., 1991; Sarosy et al., 1992). Orally administered paclitaxel, poorly absorbed due to its low solubility and efflux pump function of the drug by the multidrug efflux transporter P-gp, which is abundant in the gastrointestinal tract. Therefore, this drug is mainly used in the intravenous dosage form (Sparreboom et al., 1997). Paclitaxel has a very large distribution in the body, and is highly bound by the plasma protein, primarily albumin (95–98%) (Wiernik et al., 1987a; Wiernik et al., 1987b). In particular, it is much higher in the disposition of the liver and bile than in the other tissue (Hiroshi et al., 1994). Less than 5–10% of the administered paclitaxel was recovered as the unchanged drug in the urine of the treated patients (Wiernik et al., 1987a; Wiernik et al., 1987b; Brown et al., 1991). Paclitaxel is mainly metabolized through the liver by cytochrome P450 enzymes, especially CYP 3A and CYP 2C, 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 the paclitaxel dose, with 6α-hydroxypaclitaxel being the major metabolite (Walle et al., 1995).

In an attempt to develop safer formulations, many studies have focused on developing of a new oral formulation. However, paclitaxel is poorly absorbed when administered orally. Several investigators have reported that the poor bioavailability of paclitaxel might be result from the metabolism by cytochrome P450 enzymes or counter-transport processes by P-glycoprotein in the gut wall. In the small intestine, P-glycoprotein is co-localized at the apical membrane of the cells with cytochrome P450 (CYP 3A4) (Gottesman and Pastan, 1993). P-gp and CYP3A4 might act synergistically to the pre-systemic drug metabolism (Gan et al., 1996; Watkins, 1996; Wacher et al., 1998; Ito et al., 1999) to make the substrate of P-gp circulate between the lumen and epithelial cells, leading to prolonged exposure to CYP 3A4, resulting in a reduced absorption of the drug. Our recent study shown that paclitaxel bioavailability after oral administration of paclitaxel or prodrug to rats pretreated with quercetin was enhanced by inhibition of P-glycoprotein and CYP 3A in the intestinal mucosa (Choi et al., 2004).

The aim of this study was to investigate oral paclitaxel formulations as a paclitaxel prodrug, which will be more convenient than the i.v. dosage forms, by attempting to enhance the bioavailability of paclitaxel after the oral administration of the paclitaxel prodrugs in rats.

2. Material and methods

2.1. Materials

Paclitaxel was purchased from Samyang Genex Co. (Taejon, USA). Prodrug was obtained from Tech. Lab. in Chosun University. *N*-Butyl *p*-hydroxybenzoate (Butylparaben) were purchased from the Sigma (St. Louis, MO, USA), Saline (0.9% NaCl injectable solution) was acquired from the Choongwae Co. (Seoul, Korea). Acetonitrile, methanol, *tert*-butylmethylether and Tween80 were purchased from the Merck Co. (Darmstadt, Germany). Phosphoric acid was purchased frome Junsei Co. (Tokyo, Japan). Other chemicals were of reagent grade and were used without further purification. The apparatus were used high performance liquid chromatography (HPLC), Waters 1515 isocratic HPLC Pump, Waters 717 plus autosampler, Waters 2487 Dual absorbance detector, Waters Co. (Milford, MA, USA), a centrifugal evaporator (Rikakikai Co., Japan), a mechanical stirrer (Scientific Industries, USA), a centrifuge (Hanil Science Industrial Co., Korea), a microcentrifuge (National Labnet, USA), a sonicater (Daihan Co., Korea), a refrigerated bath circulator and a Rotamix (SeouLin Biosience, Korea).

2.2. Synthesis of prodrug

A water-soluble PEGylated paclitaxel prodrug compound was obtained by introducing a new self-immolating linker, which was spontaneously decomposed, into paclitaxel and a water-soluble polymer and

combines the water-soluble polymer with the resulting product. The prodrug compound was rapidly hydrolyzed by an esterase to generate the physiologically active paclitaxel (Jo, 2004). The prodrug, 7-mPEG 5000-succinyloxymethyloxycarbonyl-paclitaxel was synthesized as follows.

7-chloromethyloxycarbonyl-paclitaxel (1.057 m mol) was dissolved in anhydrous benzene. monomethoxypolyethyleneglycol 5000-succinate (1.057 mmol), sodium iodide (3.171 mmol), potassium carbonate (1.902 mmol) and 18-crown-6 ether (0.739 mmol) were mixed with the resulting solution. The mixture was stirred for 36 h under reflux and dried under reduced pressure to remove benzene, which was then dissolved in dichloromethane. The obtained material was filtered to remove the undissolved material. The organic layer was washed twice with water, the separated organic layer was dried using anhydrous magnesium sulfate to remove the water, dried under reduced pressure and recrystallized from isopropyl alcohol to obtain the solid material. The solid material was purified using HPLC for collection (Prep. HPLC) to yield 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.20–3.41 (m, mPEG), 5.77 (m, 2H, $J = 5.85$ Hz, OCOO$\underline{CH_2}$O), 5.42 (s, 1H, $J = 5.85$ Hz, OCOO$\underline{CH_2}$O). More detailed procedure will be appeared elsewhere (Jo, 2000; Sohn et al., 2003; Jo et al., 2003).

2.3. Animal experiments and drug administration

Male Sprague–Dawley rats (280–320 g), which were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Korea), had free access to normal standard chow diet (Jae Il 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 weighing 280–320 g were fasted for at least 24 h prior to the experiment and were given water freely. Using a 25% urethane (3 ml/kg) anesthesia, the right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling.

Paclitaxel suspension (added tween80 10 μl in distilled water 1.2 ml) and the prodrugs (87.5, 175, 350 mg/kg) were given to rats orally. The blood samples (0.6 ml) were withdrawn from the femoral artery at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after the drug administration. The plasma samples were obtained by centrifuging at 5000 rpm for 5 min. The separated 0.25 ml plasma was stored at −35 °C until HPLC analysis.

2.4. Assay and HPLC conditions

The plasma concentrations of paclitaxel were determined by HPLC assay using a modification of a method reported elsewhere (Lee et al., 1999). Briefly, 50 μl of *n*-butyl *p*-hydroxybenzoate (2 μg/ml), as the internal standard, and 4 ml of *tert*-butylmethylether were added to 0.25 ml of the plasma samples. It was then mixed on a rotamix for 20 min and centrifuged at 3000 rpm for 15 min. Three milliliter of organic layer was transferred to a clean test tube and evaporated using a centrifugal evaporator at 30 °C. The residue was then dissolved in 0.3 ml of a 0.5 g/ml $ZnSO_4$ solution [$ZnSO_4$:methanol:ethylene glycol (0.5 g:100 ml:1 ml)] and centrifuged at 5000 rpm for 5 min. Fifty microliters of the solution was injected into the HPLC system.

The HPLC system consisted of a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler, a Waters 2487 Dual λ absorbance detector (Waters Co., Milford, MA, USA) and a computing integrator. The detector wavelength was set to 227 nm and the column, a Symmetry C_{18} (4.6 mm × 150 mm, 5 μm, Waters Co., USA), was used at room temperature. Mixtures of acetonitrile: methanol: 0.5 mM phosphate buffer (pH, 3.8) (38:22:40, v/v/v) were used as the mobile phase at a flow rate of 1.2 ml/min. The retention times were as follows: internal standard, 5.3 min and paclitaxel, 7.7 min.

2.5. Pharmacokinetic analysis

The noncompartmental pharmacokinetic analysis was performed using the LAGRAN computer program (Rocci and Jusko, 1983), which employs the

LAGRAN method to calculate the AUC of the plasma concentration as a function of time. The area under the curves was calculated using the LAGRAN method. 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 the 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 (AB%) of paclitaxel after oral administration per the IV administration was calculated as follows:

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

2.6. Statistical analysis

All the means are presented with their standard deviation (Mean ± S.D.). The unpaired Student's *t*-test was used to determine the difference between the paclitaxel dose and the prodrug dose. The differences were considered to be significant at $P < 0.05$.

3. Results and discussion

The plasma concentrations of paclitaxel after the oral administration of paclitaxel (25, 50, 100 mg/kg) and the PEGylated paclitaxel prodrugs (87.5, 175, 350 mg/kg) are shown in Figs. 1 and 2. The plasma concentrations of paclitaxel by the oral prodrug were significantly ($P < 0.01$) higher than the paclitaxel as the same dose of paclitaxel. The bioavailability and pharmacokinetic parameters of paclitaxel after the oral administration of paclitaxel (25, 50, 100 mg/kg) and the prodrug (87.5, 175, 350 mg/kg) are shown in Tables 1 and 2.

The area under the plasma concentration–time curve (AUC) of paclitaxel by the oral paclitaxel (25, 50, 100 mg/kg) were 836 ± 227, 1602 ± 419 and 3076 ± 758 ng/ml h, the relative bioavailability of paclitaxel (RB%) were 100, 192 and 368,

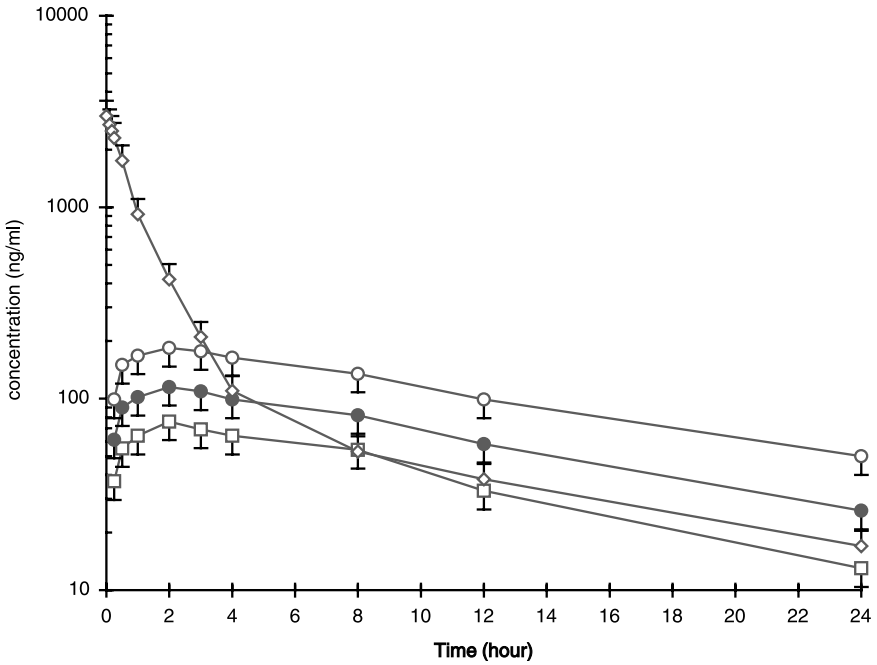

Fig. 1. Mean plasma concentration–time curves of paclitaxel after the oral administration of paclitaxel (25 mg/kg, 50 mg/kg, 100 mg/kg)) and IV (2 mg/kg) in rats ($n = 8$), The bar represents standard deviation: (□) paclitaxel 25 mg/kg; (●) paclitaxel 50 mg/kg; (○) paclitaxel 100 mg/kg; (◇) paclitaxel IV 2 mg/kg.

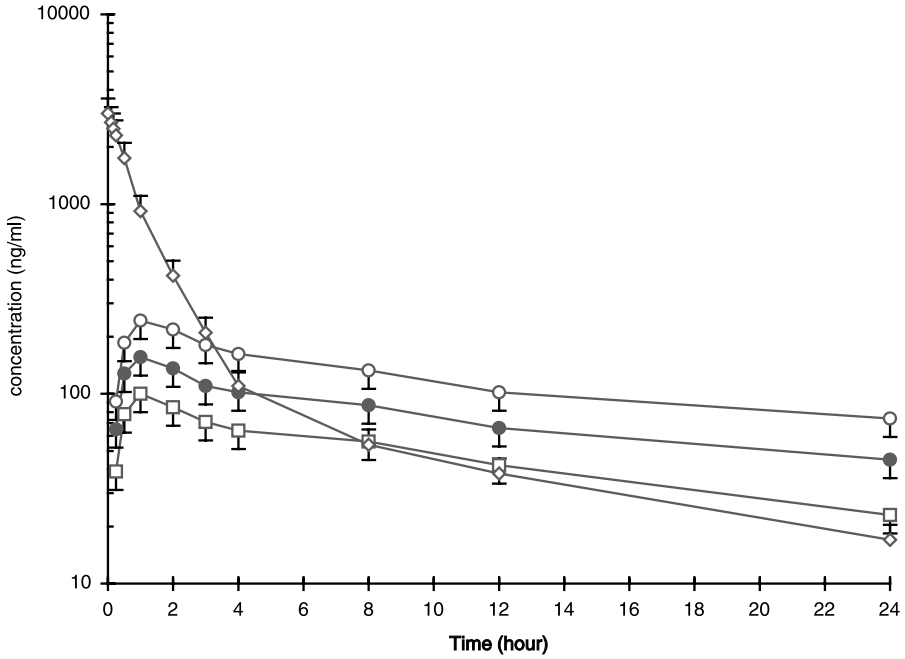

Fig. 2. Mean plasma concentration–time curves of paclitaxel after the oral administration of prodrug (87.5 mg/kg, 175 mg/kg, 350 mg/kg) and IV (2 mg/kg) in rats ($n = 8$), The bar represents standard deviation: (□) prodrug 87.5 mg/kg; (●) prodrug 175 mg/kg; (○) prodrug 350 mg/kg; (◇) paclitaxel IV 2 mg/kg.

which were increased dose-dependently ($P < 0.006$, $r = 0.9996$). The AUC of paclitaxel by the prodrug (87.5, 175, 350 mg/kg) were 1646 ± 418, 3079 ± 709 and 5998 ± 1538 ng/ml h, RB% of paclitaxel from the prodrug were 100, 187 and 364, also increased dose-dependently ($P < 0.003$, $r = 0.003$). The AUC of paclitaxel after the intravenous administration (2 mg/kg) was 3992 ± 902 ng/ml h. The mean absolute bioavailability (AB%) of paclitaxel was 1.6% by the oral paclitaxel. AB% of oral prodrug was 6.3%, which increased 3.94-fold compared to paclitaxel parent drug in rats. The peak plasma concentration of paclitaxel (C_{max}) by the oral paclitaxel (25, 50, 100 mg/kg) was 61 ± 16, 104 ± 27 and 166 ± 41 ng/ml. The peak plasma concentration of paclitaxel (C_{max}) by the prodrug (87.5, 175, 350 mg/kg) were 114 ± 28, 180 ± 46 and 339 ± 84 ng/ml. The peak plasma concentration of paclitaxel in the prodrug, 350 mg/kg (50 mg/kg as paclitaxel), was significantly higher ($P < 0.01$) than paclitaxel 50 mg/kg. The time to reach

Table 1
Mean (±S.D.) pharmacokinetic parameters of paclitaxel after the oral administration of paclitaxel (25, 50, 100 mg/kg) in rats

Parameters	25 mg/kg	50 mg/kg	100 mg/kg	IV 2 mg/kg
AUC (ng/ml h)	836 ± 227	1602 ± 419*	3076 ± 758**	3992 ± 902
C_{max} (ng/ml)	61 ± 16	104 ± 27*	166 ± 41**	–
T_{max} (h)	2.0 ± 0.6	2.0 ± 0.6	2.0 ± 0.5	–
$t_{1/2}$ (h)	9.1 ± 2.4	9.8 ± 2.5	10.1 ± 2.5	8.4 ± 2.1
AB (%)	1.7	1.6	1.6	100
RB (%)	100	192*	368**	

Each value represents the mean ± S.D. ($n = 8$), *$P < 0.05$, **$P < 0.01$, significant difference compared to 25 mg/kg of paclitaxel. AUC: area under the plasma concentration–time curve from time zero to time infinity. C_{max}: maximum plasma concentration. T_{max}: time of C_{max}. $t_{1/2}$: terminal half-life. AB: absolute bioavailability. RB: AUC rate compared to AUC control.

Table 2
Mean (±S.D.) pharmacokinetic parameters of paclitaxel after the oral administration of the PEGylated paclitaxel prodrug (87.5, 175, 350 mg/kg) and IV (2 mg/kg) in rats

Parameters	87.5 mg/kg	175 mg/kg	350 mg/kg	IV 2 mg/kg
AUC (ng/ml h)	1646 ± 418	3079 ± 709*	5998 ± 1538**	3992 ± 902
C_{max} (ng/ml)	114 ± 28	180 ± 46*	339 ± 84**	–
T_{max} (h)	1.4 ± 0.25	1.4 ± 0.28	1.5 ± 0.38	–
$t_{1/2}$ (h)	12.0 ± 3.4	15.1 ± 3.6	15.7 ± 4.1	8.4 ± 2.1
AB (%)	6.6	6.2	6.1	100
RB (%)	100	187*	364**	–

Each value represents the mean ± S.D. ($n = 8$), $^{*}P < 0.05$, $^{**}P < 0.01$, significant difference compared to 87.5 mg/kg of prodrug. AUC: area under the plasma concentration–time curve from time zero to time infinity, $C_{max:}$ maximum plasma concentration. T_{max}: time of C_{max}. $t_{1/2}$: terminal half-life. AB: absolute bioavailability. RB: AUC rate compared to AUC control.

the maximum plasma concentration (T_{max}), the terminal half-life ($t_{1/2}$) of paclitaxel in the paclitaxel control (25, 50, 100 mg/kg) showed no apparent changes compared to those of the prodrug.

This study introduced a water-soluble PEGylated paclitaxel prodrug compound, 7-mPEG 5000-succinyloxymethyloxycarbonyl-paclitaxel, which was obtained by introducing a new self-immolating linker that is spontaneously decomposed into paclitaxel combining a water-soluble polymer (Jo, 2000; Jo, 2004; Jo et al., 2003; Sohn et al., 2003). The molecular weight of paclitaxel and the prodrug is approximately 700 and 5000, respectively. At the same dose of paclitaxel, the AUC of paclitaxel in the 350 mg of prodrug (50 mg as paclitaxel) resulted in a remarkable increase compared to 50 mg of paclitaxel. Therefore, the mean absolute bioavailability of prodrug was 3.9-fold higher than paclitaxel.

It was reported that paclitaxel is metabolized by cytochrome p-450 (CYP3A) both in the liver and in the epithelial cells of the small intestine, and in addition, the absorption of paclitaxel was inhibited by the P-gp efflux pump in the intestinal mucosa (Sparreboom et al., Cresteil et al., 1994; Kumar et al., 1994; Rahman et al., 1994; Sonnichsen et al., 1995). The increased AB% of paclitaxel by prodrug might have resulted from the physicochemical properties of the prodrug, which is a water-soluble compound and permeate through the gastrointestinal mucosa more easily than paclitaxel without obstruction of P-gp and cytochrome P-450 in the gastrointestinal mucosa (Jo et al., 2003; Sohn et al., 2003; Choi et al., 2004). It can be rapidly hydrolyzed by an esterase to generate the physiologically active paclitaxel (Sohn et al., 2003), and leads to a high concentration of paclitaxel in the plasma to make the higher bioavailability than the parent drug.

The AB% of paclitaxel in the prodrug was 6.3, which is enough to be administered orally as a prodrug. Based on these results, it might be feasible to develop oral paclitaxel preparations, which is more convenient than the i.v dosage forms.

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