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Pharmacokinetics and Oral Bioavailability of Paclitaxel Microemulsion in Rats

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ABSTRACT – The objectives of this study were to formulate oral paclitaxel microemulsion and to compare the bioavailability of paclitaxel in the microemulsion formulation from the commercially available Taxol® formulation. Paclitaxel microemulsion was formulated with much less amount of Cremophor EL™ as compared with Taxol® to reduce severe adverse reactions produced by Cremophor EL™. The area under the plasma concentration-time curve from 0 hr to 24 hr (AUC_{0-24}), maximum plasma concentration (C_{max}), and relative bioavailability of palcitaxel microemulsion were increased as compared with Taxol® after oral administration. The time required to reach C_{max} (T_{max}) of palcitaxel microemulsion was significantly shorter than Taxol® following oral administration. These results suggest the faster intestinal absorption and the enhanced oral bioavailability of paclitaxel in the microemulsion formulation.

Key words – Paclitaxel microemulsion, Pharmacokinetics, Oral bioavailability

Paclitaxel derived from the bark of the Pacific yew tree (*Taxus brevifolia*) is an anticancer drug,[1,2] which has been used to treat refractory ovarian, breast, and non-small cell lung cancers.[3,4]

The currently marketed IV dosage form of paclitaxel (Taxol®) has been formulated with a mixture of Cremophor EL™ and dehydrated ethanol (1:1, v/v) to dissolve paclitaxel completely. However, Cremophor EL™ has been reported to produce severe toxicities, such as nephrotoxicity, neurotoxicity and hypersensitivity reactions mediated by endogenous histamine release.[5,6] Moreover, the content of Cremophor EL™ in Taxol® is much higher than that in cyclosporine and teniposide formulations.[7] It has been known that most side effects of Taxol® are caused by Cremophor EL™ rather than paclitaxel itself.

Numerous studies have been progressed to develop oral formulations for safe clinical application. However, paclitaxel is only administered intravenously because of its poor bioavailability. Oral bioavailability of paclitaxel has been reported to be less than 1%.[8] Its poor bioavailability is due, at least in part, to poor solubility, metabolism by cytochrome P-450 existed in both liver and small intestine, and efflux from epithelial cells of small intestine by P-glycoprotein.[9,10]

A microemulsion formulation has been reported to improve the rate and extent of absorption of liphophilic compounds.[11-16] Microemulsions are thermodynamically stable dispersions of water and oil, which possess many advantages, such as clarity, homogeneity, and ease of preparation.[16]

In the present study, paclitaxel microemulsion was formulated for oral administration and the bioavailability of paclitaxel in the microemulsion was compared with that in Taxol®.

Materials and Methods

Materials

Paclitaxel was purchased from Samyang Co. (Seoul, Korea) and 4-hydroxybenzoic acid n-hexyl ester (internal standard) was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Phosphoric acid was supplied by Showa chemical (Tokyo, Japan) and diethyl ether was purchased from Duksan pure chemical (Ansan, Korea). Acetonitrile and methanol were supplied by Merk (Darmastadt, Germany). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Animals

Male Sprague-Dawley rats (7~8 weeks old) were purchased from Samtako Bio Korea (Kyunggi-Do, Korea). Rats were housed in a light controlled room kept at a temperature of 23 ± 3°C and a relative humidity of 50 ± 5% with free access to a normal chow diet and water.

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Preparation of microemulsion formulation

Transcutol-P, polyethyleneglycol 200, Tween® 80, Cremophor EL™, Cremophor RH40, *dl*-α-tocopheryl acetate and ethyl linoleate were mixed for 10 min at room temperature, followed by homogenization for 10 min at 45 ± 5°C. Then, paclitaxel was added to the homogenized vehicle, followed by vortexing for 10 min at room temperature, homogenization for 10 min at 45 ± 5°C, and sonication for 2 min at room temperature.

Animal experiments and Pharmacokinetic analysis

Male Sprague-Dawley rats, weighing 230~250 g, fed on a normal chow diet and were allowed free access to water for at least one week before the experiments. The common carotid artery of the rats was cannulated with polyethylene tubing (PE-60) under the light anesthesia using diethyl ether for blood sampling. The rats were housed individually for a day to allow them to recover from surgery.

The rats were fasted overnight with free access to water before the experiments. The rats were assigned to one of three groups. A commercial formulation of paclitaxel (Taxol®) was injected intravenously at a dose of 2 mg/kg or administered orally at a dose of 25 mg/kg to the rats. This formulation was prepared by dissolving paclitaxel in Cremophor EL and ethanol (1:1, v/v) to a concentration of 6 mg/mL, followed by dilution with isotonic saline to a final concentration of 2 mg/mL just before use. Paclitaxel microemulsion formulation was also given to the rats orally at a dose of 25 mg/kg. The microemulsion formulation was prepared to a concentration of 25 mg/mL, which was diluted with deionized water to a final concentration of 8.3 mg/mL just before use.

After IV injection, blood samples (0.25 mL) were taken from the carotid artery at 0, 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 10 and 24 hr. About 0.25 mL of blood was collected via the carotid artery at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 10 and 24 hr after oral administration. The plasma was collected after centrifugation of the blood and stored at −20°C until HPLC analysis.

Pharmacokinetic parameters were estimated from plasma concentration-time curve using WinNonlin software (Pharsight Co., Mountain View, CA., USA). The pharmacokinetic parameters estimated from the data were as follows; the area under the plasma concentration-time curve from 0 hr to 24 hr (AUC_{0-24}), elimination half-life ($t_{1/2}$), volume of distribution (V_d) and clearance (Cl_t). The maximum plasma concentration (C_{max}) and the time required to reach C_{max} (T_{max}) were directly determined from the individual plasma concentration-time profiles. Absolute bioavailability (AB, %) was calculated by the ratio of AUC_{0-24} obtained following oral administration to that following intravenous injection with normalization for dose. Relative bioavailability (RB, %) was calculated by the ratio of AUC_{0-24} of paclitaxel microemulsion to that of Taxol®.

HPLC analysis

The plasma concentrations of paclitaxel were determined with slight modification of the HPLC method reported by Lee *et al.*[17] The HPLC system used for this study was an Agilent HP1100 series system, which consisted of a model 1100 quaternary pump with degasser pump, a model 1100 variable wavelength detector, a model 1100 thermostatted auto-sampler and a model HPLC 2D agilent ChemStation software. Capcellpak C_{18} UG120 column (4.6 mm × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was used as an analytical column and Symmetry C_{18} column (3.9 mm × 20 mm, 5 μm, Waters, Ireland) was used as a guard column. The mobile phase consisting of acetonitrile and 0.1% phosphoric acid (51:49, v/v) was run at 1.3 mL/min and the effluent was detected at 227 nm using a UV-detector.

To 100 μL of rat plasma, 25 μL of *n*-hexyl *p*-hydroxy benzoic acid (2.5 μg/mL, I.S.) was added and the mixture was extracted with 1.6 mL of *tert*-butyl methyl ether. After centrifugation at 3000 rpm for 15 min, a 1.4 mL organic layer was transferred to a clean tube and evaporated in a centrifugal evaporator for 30 min at 40°C. The residue was then dissolved in 70 μL of 60% acetonitrile in deionized water by vortexing for 1 min. A 50 μL of the solution was injected into the HPLC system.

Statistical analysis

All the results were expressed as mean ± standard deviation. An unpaired Student's *t*-test was used to evaluate any significant difference between Taxol® and paclitaxel microemulsion groups. The differences were considered to be significant when p value was less than 0.05.

Results and Discussion

Characteristics of paclitaxel microemulsion

Paclitaxel microemulsion was thermodynamically stable dispersions of water and oil with transparent appearance and homogeneity. This formulation with a paclitaxel concentration of 70 mg/mL was stable more than a month at room temperature.

HPLC validation

Paclitaxel and I.S. were eluted at 8.9 and 16.9 min in rat

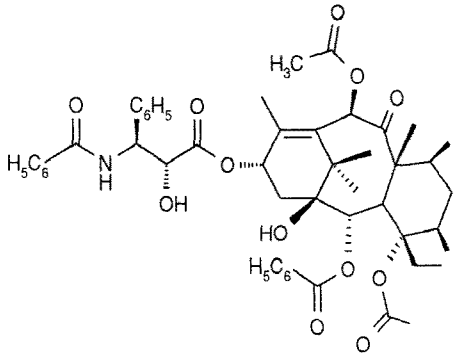

Figure 1–Structure of paclitaxel (5, 20-epoxy-1, 2α, 4, 7β, 13α-hexahydroxytax-11-en-9-one4, 10-diacetate 2- benzoatel 3-ester with (2R,3S)-N-benzoyl-3-phenyllisoserine).

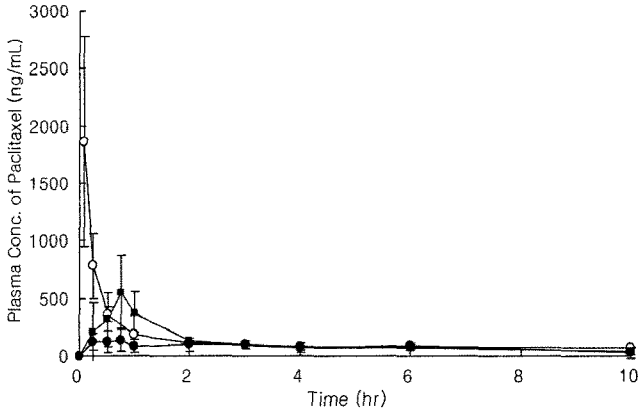

Figure 2–Mean plasma concentration-time curves of paclitaxel following oral administration of Taxol® or paclitaxel microemulsion and IV injection of Taxol® in rats. Bars represent the S.D. (n=4-5). (○) IV injection of Taxol® (2 mg/kg), (●) oral administration of Taxol® (25 mg/kg), (■) oral administration of paclitaxel microemulsion (25 mg/kg).

plasma, respectively, and well separated from endogenous interfering substances (data not shown). The calibration curve was linear over the concentration range from 0.01 to 50 mg/mL with straight-line regression equation, $y=0.5087\times-0.0186$ ($R^2=1.000$), where y is the peak area ratio of paclitaxel to I.S. and x is the concentration of paclitaxel. The intra- and inter-day precision was varied between 6.29 and 12.7%, and 7.19 and 14.3%, respectively. The intra- and inter-day accuracy was in the range from 99.5 to 113%, and 90.2 to 115%, respectively. The analytical method was valid in terms of specificity, linearity, precision and accuracy.

Pharmacokinetics of paclitaxel microemulsion

Mean plasma paclitaxel concentration-time curves following oral administration of Taxol® or pacltaxel microemulsion and IV injection of Taxol® are shown in Figure 1. The pharmacokinetic parameters of paclitaxel estimated from plasma paclitaxel concentration-time profiles are shown in Table I.

Mean $AUC_{0\text{-}24}$ and C_{max} of the paclitaxel microemulsion were approximately 2-fold and 5-fold greater than those of Taxol® following oral administration, respectively. The T_{max} was significantly decreased in the paclitaxel microemulsion as compared with Taxol® ($p<0.05$). In addition, relative bioavailability of paclitaxel microemulsion (171%) was greater than that of Taxol® after oral administration.

Based on the results of pharmacokinetic study, paclitaxel microemulsion formulated in this study might have advantages over Taxol® formulation. The amount of Cremophor EL™, which can produce severe side effects, such as hypersensitivity reactions, nephrotoxicity and neurotoxicity, in the microemulsion formulation was significantly reduced to be less than 1/6. It has been reported that the Cremophor EL is an important limiting factor for the absorption of orally administered paclitaxel from the intestinal lumen due to the entrapment of pacli-

Table I–*Pharmacokinetic Parameters of Paclitaxel Following Oral Administration of Taxol® or Paclitaxel Microemulsion and IV Injection of Taxol® in Rats*

PK Parameters	Paclitaxel microemulsion (25 mg/kg, PO)	Taxol® (25 mg/kg, PO)	Taxol® (2 mg/kg, IV)
C_{max} (ng/mL)	513.7±273.4*	112.8±28.1	-
T_{max} (hr)	0.8±0.3**	2.5±0.6	-
$t_{1/2}$ (hr)	13.1±7.1	17.6±6.8	8.1±1.5
V_d (mL)	-	-	2370.0±247.0
Cl_t (mL/hr)	-	-	211.0±51.3
$AUC_{0\text{-}24}$ (ng·hr/mL)	1018.0±391.5	592.7±199.3	1427.0±387.5
AB (%)	5.7	3.3	
RB (%)	171	100	

Each value was presented as mean ± S.D. (n=4-5).
*$p<0.05$ compared with Taxol®
**$p<0.01$ compared with Taxol®

taxel in micelles and protein binding.[3,18] In addition, numerous studies have reported that microemulsion system improved the rate and extent of absorption, and the overall bioavailability of lipid-soluble drugs.[11-16,19] In agreement with previous reports, AUC_{0-24}, C_{max} and relative bioavailability of paclitaxel microemulsion were increased as compared with Taxol®. In addition, the T_{max} of paclitaxel microemulsion was shorter than Taxol® (0.8 ± 0.3 hr vs. 2.5 ± 0.6 hr), suggesting the faster absorption of paclitaxel from the microemulsion formulation into the intestine.

In conclusion, paclitaxel microemulsion was formulated with much less amount of Cremophor EL™ (about 1/6) as compared with Taxol®. The AUC_{0-24}, C_{max} and relative bioavailability of palcitaxel microemulsion were greater than Taxol® while the T_{max} of that was shorter than Taxol® following oral administration, suggesting the enhanced oral absorption of paclitaxel in the microemulsion formulation.

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