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Comparative protein binding of Taxotere and SID530, a new docetaxel formulation with hydroxypropyl-beta-cyclodextrin, in human plasma *in vitro*

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The purpose of this study was to evaluate the plasma-protein binding of docetaxel in two different formulations, Taxotere and SID530, a new docetaxel formulation with hydroxypropyl-beta-cyclodextrin (HP-β-CD), in human plasma *in vitro,* using equilibrium dialysis. Unbound docetaxel concentration in the human plasma was determined by LC-MS/MS analysis. SID530 showed a plasma-protein binding profile comparable to that of Taxotere in the clinically relevant concentration range of docetaxel. In both formulations, the unbound fraction of docetaxel increased in a concentration-dependent biphasic manner. The resulting data indicate that the excipient used in SID530, HP-β-CD, generates similar effects as polysorbate 80 of Taxotere in terms of plasma-protein binding of docetaxel.

1. Introduction

Docetaxel is an antimitotic chemotherapy medication mainly used in the treatment of breast, ovarian, prostate, and non-small-cell lung cancer (Clarke and Rivory 1999; Lyseng-Williamson and Fenton 2005; Michael et al. 2009). It is sold under the brand name Taxotere, which is a polysorbate-80 formulation for intravenous administration.

Recently, a new formulation of docetaxel, SID530, was developed by SK chemicals (Seongnam, Korea). SID530 uses the complexing agent hydroxypropyl-beta-cyclodextrin (HP-β-CD) as a pharmaceutical formulation vehicle along with a water-soluble polymeric stabilizer, povidone K12. HP-β-CD generally increases the solubility of insoluble compounds by complex formation (Gould and Scott 2005). In the case of SID530, several molecules of HP-β-CD may encircle the relatively large docetaxel molecule to form a higher order noninclusion complex or aggregate and facilitate the dissolution of the drug (Brewster and Loftsson 2007). SID530 is expected to improve chemical and physical stability of the active pharmaceutical ingredient compared with Taxotere and easy to handle and prepare as existing as crystalline powder. Moreover, this new formulation reduces the side effects of Taxotere caused by polysorbate 80.

It is supposed that a 5-mL parenteral injection of a drug–HP-β-CD solution in humans would generally result in complete dissociation of the drug from the HP-β-CD complex (Stella and He 2008). Therefore, the administered SID530 may readily release the free fraction of docetaxel, which subsequently equilibrates with binding plasma proteins. However, the HP-β-CD used in SID530 could have a different form of interaction with the drug from the polysorbate 80 in Taxotere and consequently lead to a different pharmacokinetic behavior of docetaxel *in vivo*.

Docetaxel binds extensively to plasma proteins including α1-acid glycoprotein, albumin, and lipoproteins (Urien et al. 1996) with a plasma-protein binding rate ranging from 92% to 96% (Loos et al. 2003). As unbound docetaxel, rather than total drug concentration, may better correlate with treatment outcomes (i.e., toxicity and efficacy) (Baker et al. 2005), changes in the plasma-protein binding property may affect the pharmacokinetics of docetaxel and, consequently, the efficacy and toxicity of the drug. Notably, it has been reported that the plasma binding of docetaxel could be influenced by polysorbate 80, which is used as the pharmaceutical formulation vehicle in Taxotere. In this context, the equivalence of the plasma-protein binding property should be addressed in order to assure bioequivalence between Taxotere and the new docetaxel formulation with a different excipient.

In this study, we investigated the plasma-protein binding of docetaxel in human plasma for two different formulations with different excipients, namely, Taxotere and SID530, in an effort to predict their bioequivalence. The plasma-protein binding of docetaxel was determined *in vitro* using equilibrium dialysis and LC-MS/MS analysis.

2. Investigations, results, and discussion

2.1. LC-MS/MS analysis of docetaxel

The LC-MS/MS method for the determination of docetaxel in human plasma was developed and validated with regard to specificity, linearity, accuracy, and sensitivity. Docetaxel and paclitaxel (IS) were eluted at 2.60 and 2.67 min, respectively. No interfering peak was observed in the samples at the retention times of either docetaxel or paclitaxel. The calibration curves were constructed by plotting peak area ratios of docetaxel/paclitaxel (IS) against docetaxel concentrations and

Table 1: Intra- and inter-day accuracy and precision for the LC-MS/MS analysis of docetaxel in human plasma

Quality control concentration (ng/mL)	Intra-day ($n = 5$)		Inter-day ($n = 5$)	
	Accuracy (%)	Precision (RSD,%)	Accuracy (%)	Precision (%RSD)
10	119.0	8.9	88.3	15.5
300	96.7	3.6	96.7	9.4
3000	87.5	8.5	87.7	11.9

measured using linear least-squares regression analysis. The calibration curve for docetaxel showed a good linearity over the concentration range of 10∼3000 ng/mL with a correlation coefficient (r) > 0.99. Precision and accuracy were determined by repeated analysis of three concentration levels of QC samples (10, 300, and 3000 ng/mL, $n = 5$) for five consecutive days. The accuracy (as % recovery) was 87.5∼119.0% and the precision (as % relative standard deviation) was less than 15.5% for all samples tested (Table 1).

2.2. *Comparative plasma protein binding of SID530 and Taxotere*

The percentages of unbound docetaxel in human plasma for Taxotere and SID530 were determined using equilibrium dialysis. The clinically relevant concentration range of docetaxel is 1 to 5 μg/mL. However, a higher concentration range up to 20 μg/mL was investigated in order to reflect the initial phase concentration after an intravenous infusion.

The F_u values of docetaxel in the two formulations measured by equilibrium dialysis are listed in Table 2. In the case of Taxotere, the F_u of docetaxel was 4.7∼5.9% at lower concentrations (1, 2, and 5 μg/mL), but gradually increased to 7.9% as the docetaxel concentration increased to 20 μg/mL. The F_u of docetaxel in SID530 was 5.7∼6.0% at the concentration range of 1∼10 μg/mL. At a higher concentration (20 μg/mL), the F_u was similar to that of Taxotere. The plasma-protein binding rate of docetaxel has been reported to range from 92% to 96% depending on the composition of the plasma proteins, particularly the α1-acid glycoprotein concentration (Urien et al. 1996). Therefore, this result is comparable to that of previous reports.

The present result revealed an increase in the F_u in a concentration-dependent biphasic manner. This pattern has been reported previously by Loos et al. (2003), who suggested that the biphasic pattern was caused by the excipient polysorbate 80 used in Taxotere, and the fraction of unbound docetaxel increased abruptly at polysorbate 80 concentrations above 0.25 μL/mL. In our data, the F_u of Taxotere increased at a concentration of 10 μg/mL docetaxel, which is equivalent to a polysorbate 80 concentration of 0.25 μL/mL. This is in agreement with the previous result by Loos et al. (2003). In the case of SID530, the fraction of unbound docetaxel was a little bit higher than that of Taxotere at lower concentrations and an increase in the F_u was observed at a concentration of 20 μg/mL docetaxel. However, the values between the two groups were not statistically different at any concentration tested.

These data indicate that the excipient used in SID530 HP-β-CD generates similar effects on the plasma-protein binding of docetaxel as polysorbate 80 of Taxotere, although the underlying mechanism is yet to be determined.

3. Experimental

3.1. *Materials and reagents*

SID530 was obtained from SK Chemicals (Seongnam, Korea), and Taxotere was purchased from Sanofi-Aventis (Paris, France). Human plasma was purchased from Innovative Research (Novi, MI, USA). The rapid equilibrium dialysis (RED) plate was purchased from Thermo Scientific (Rockford, IL, USA). Docetaxel (analytical standard compound), sulfaphenazole (internal standard), and 10 × phosphate buffered saline pH 7.4 were purchased from Sigma-Aldrich Corporation (St. Louise, Mo, USA). Dextrose (5% in water) was purchased from Daihan Pharm (Ansan, Korea). Other chemicals used were of reagent or HPLC grade.

3.2. *Test solution preparation*

The frozen dry powder of SID530 in a vial (docetaxel 20 mg per vial) was reconstituted with distilled water to a concentration of 10 mg/mL. The reconstituted solution was diluted with 5% dextrose solution to a final concentration of 0.4 mg/mL. In the case of Taxotere, docetaxel dissolved with polysorbate 80 in a vial (20 mg per vial) was reconstituted with 13% ethanol to a pre-mix solution and then diluted with 5% dextrose solution to a final concentration of 0.4 mg/mL. The docetaxel concentration of each sample was confirmed by HPLC analysis (data not shown).

3.3. *Equilibrium dialysis*

Each test-drug solution was added to human plasma to yield test plasma samples of various concentrations (1, 2, 5, 10, and 20 μg/mL) of docetaxel. The test plasma sample (200 μL) and PBS (200 μL) were added to the plasma and buffer chambers, respectively, and incubated at 37 °C for 6 h in a shaking water bath. The plasma sample and buffer solution (50 μL each) were then transferred from their respective chambers to microcentrifuge tubes and used for the LC-MS/MS analysis. The percentage of unbound docetaxel was calculated as follows:

% unbound = (concentration in the buffer chamber/concentration in the plasma chamber) × 100

3.4. *Sample preparation*

The collected samples (50 μL each) from the plasma-protein binding assays were spiked with 10 μL of the internal standard solution (IS, 6 μg/mL paclitaxel in DMSO) and 500 μL of ethyl acetate. The mixtures were vortex-mixed for 30 s, for liquid-liquid extraction, followed by centrifugation at

Table 2: Extent of docetaxel binding to human plasma proteins

Formulation	Fraction unbound (%, mean ± SD)				
	Docetaxel concentration (μg/mL)				
	1	2	5	10	20
Taxotere (Polysorbate 80 conc., μL/mL)	4.7 ± 1.1 (0.025)	4.7 ± 0.6 (0.05)	5.9 ± 0.7 (0.125)	7.5 ± 0.7 (0.25)	7.9 ± 0.3 (0.5)
SID530	5.7 ± 0.4	5.9 ± 0.3	5.8 ± 0.6	6.0 ± 0.7	7.5 ± 0.5
P value [a]	0.299	0.066	0.834	0.099	0.511

[a] The student's t-test was conducted to assess the differences between two groups. $P < 0.05$ value was considered statistically significant.

12,000 rpm for 5 min. The supernatant (400 μL) was transferred to a microtube and evaporated using nitrogen gas. The residue was reconstituted with 400 μL of acetonitrile and then analyzed. For the preparation of calibration standard or quality control samples, docetaxel standards (5 μL) were appropriately added to 45 μL blank human plasma and prepared in the same manner as described above.

3.5. LC-MS/MS analysis

The LC-MS/MS system consisted of a Shiseido Nanospace SI-2 (Shiseido Co. Ltd., Tokyo, Japan) and an API4000 triple-quadrupole mass spectrometer (AB MDS Sciex, Toronto, Canada). The HPLC mobile phases consisted of 10 mM ammonium formate buffer (pH 4.0) (solvent A) and acetonitrile (solvent B). Chromatographic separation was achieved on a CAPCELL PAK MG C_{18} (2.0 × 50 mm, 5 μm; Shiseido, Tokyo, Japan) by using a linear gradient elution with solvents A and B (60/40∼10/90, v/v) at a flow rate of 0.3 mL/min. The run time was 10 min. Electrospray ionization was performed in a positive mode. Multiple reaction monitoring (MRM) detection was employed. The transitions monitored were 808→527 for docetaxel and 854→509 for paclitaxel (IS). The LC-MS/MS method was validated with regard to specificity, linearity, accuracy, and sensitivity according to the US Food and Drug Administration's "Guidance for Industry, Bioanalytical Method Validation, 2001."

References

Baker SD, Li J, ten Tije AJ, Figg WD, Graveland W, Verweij J, Sparreboom A (2005) Relationship of systemic exposure to unbound docetaxel and neutropenia. Clin Pharmacol Ther 77: 43–53.

Brewster ME, Loftsson T (2007) Cyclodextrins as pharmaceutical solubilizers. Adv Drug Deliv Rev 59: 645–666.

Clarke SJ, Rivory LP (1999) Clinical pharmacokinetics of docetaxel. Clin Pharmacokinet 36: 99–114.

Gould S, Scott RC (2005) 2-Hydroxypropyl-b-cyclodextrin (HP-b-CD): A toxicology review. Food Chem Toxicol 43: 1451–1459.

Loos WJ, Baker SD, Verweij J, Boonstra JG, Sparreboom A (2003) Clinical pharmacokinetics of unbound docetaxel: role of polysorbate 80 and serum proteins. Clin Pharmacol Ther 74: 364–371.

Lyseng-Williamson KA, Fenton C (2005) Docetaxel: a review of its use in metastatic breast cancer. Drugs 65: 2513–2531.

Michael A, Syrigos K, Pandha H (2009) Prostate cancer chemotherapy in the era of targeted therapy. Prostate Cancer Prostatic Dis 12: 13–16.

Stella VJ, He Q (2008) Cyclodextrins. Toxicol Pathol 36: 30–42.

Urien S, Barré J, Morin C, Paccaly A, Montay G, Tillement JP (1996) Docetaxel serum protein binding with high affinity to alpha 1-acid glycoprotein. Invest New Drugs 14: 147–151.