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Interaction of Docetaxel ("Taxotere") with Human P-Glycoprotein

Katsuro Shirakawa,[1] Kohji Takara,[2] Yusuke Tanigawara,[3] Nobuo Aoyama,[1] Masato Kasuga,[1] Fusao Komada,[2,5] Toshiyuki Sakaeda[2] and Katsuhiko Okumura[2,4]

[1]Second Department of Internal Medicine and [2]Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017 and [3]Department of Hospital Pharmacy, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582

The interaction of docetaxel ("Taxotere") with P-glycoprotein (P-gp) was examined using porcine kidney epithelial LLC-PK_1 and LLC-GA5-COL150 cells, overexpressing human P-gp selectively on the apical plasma membrane by transfection of human *MDR1* cDNA into the LLC-PK_1 cells. The basal-to-apical transport of [^{14}C]docetaxel in LLC-GA5-COL150 cells significantly exceeded that in LLC-PK_1 cells, but the apical-to-basal transport was decreased in LLC-GA5-COL150 cells. The intracellular accumulation after its basal or apical application to LLC-GA5-COL150 cells was 4- to 20-fold lower than that of LLC-PK_1 cells. Multidrug resistance (MDR) modulators, i.e., cyclosporin A and SDZ PSC 833, inhibited the basal-to-apical transport and increased the apical-to-basal transport of [^{14}C]docetaxel in LLC-GA5-COL150 cells, but verapamil affected only apical-to-basal transport. The intracellular accumulation after basal or apical application to LLC-GA5-COL150 cells was also increased by these three MDR modulators. These observations demonstrated that docetaxel is a substrate for human P-gp, suggesting that docetaxel-drug interactions occur via P-gp. The inhibition of [^{14}C]docetaxel transport by the MDR modulators, as well as daunorubicin and vinblastine, was also found in LLC-PK_1 cells, which endogenously express P-gp at lower levels, and concentrations showing similar levels of inhibition were lower than those in the case of LLC-GA5-COL150 cells. These observations indicate that it is necessary to consider the pharmacokinetic and pharmacodynamic interactions of docetaxel via P-gp.

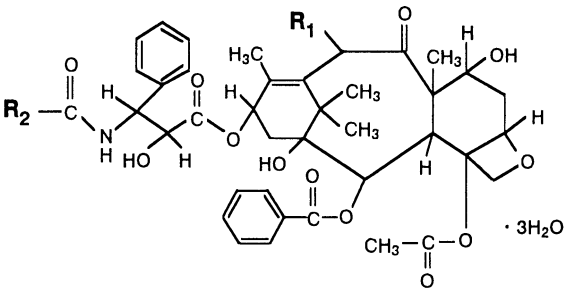
Key words: Docetaxel — "Taxotere" — P-Glycoprotein — Multidrug resistance — Transport

Docetaxel ("Taxotere") [(2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-*tert*-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate] ($C_{43}H_{53}NO_{14}\cdot 3H_2O$ (861.94); Fig. 1), a semi-synthetic analog of paclitaxel ("Taxol"), was developed in 1981. It promotes microtubule polymerization leading to cell cycle arrest at G2/M, apoptosis and finally cytotoxicity.[1,2] Tubulin normally present in cells is in dynamic equilibrium between tubulin dimers and microtubules. *Vinca* alkaloids and colchicine inhibit tubulin polymerization and thus lead to disruption of the mitotic spindle.[3] Docetaxel has been found to be superior to paclitaxel in a number of experimental models and this can be explained by improved cellular uptake and microtubule stabilization.[2] Docetaxel has significant activity in breast, non-small cell lung, ovarian and head and neck cancers.[2,4–7] Docetaxel has undergone phase I studies in a number of dosage regimens mainly in North America, Europe and Japan, and has already been approved in these countries for use in treatment of breast and non-small cell lung cancer.[8]

Anticancer drugs are usually applied in combination with other anticancer drugs to ensure higher efficacy. Anticancer drugs which have been used in combination with docetaxel include cisplatin, carboplatin, 5-fluorouracil, doxorubicin, gemcitabine, etoposide, cyclophosphamide, ifosfamide, topotecan and irinotecan.[2] In combination therapy, drug-drug interaction may arise as the result of altered pharmacokinetics and pharmacodynamics of the drugs involved. However, no significant pharmacokinetic drug-drug interactions with docetaxel have been reported, with the exception of doxorubicin[9] and etoposide,[10] and actually little information is available regarding pharmacodynamic drug-drug interactions involving docetaxel.

Previously, we established LLC-GA5-COL150 cells[11,12] selectively overexpressing human P-glycoprotein (P-gp), which is a key protein in the excretion of xenobiotics and the multidrug resistance (MDR) of tumor cells, on the apical membrane by transfection of *MDR1* cDNA into porcine kidney epithelial LLC-PK_1 cells. This cell line has been shown to form monolayers with tight junctions, and has been used for direct assessment of P-gp-mediated drug transport.[11–15] Using this cell line, we demonstrated the transport of vinblastine and doxorubicin via P-gp and the

[4] To whom correspondence should be addressed.
E-mail: okumurak@kobe-u.ac.jp
[5] Present address: Department of Drug Informatics, Faculty of Pharmaceutical Sciences, Josai University, Keyakidai, Sakado, Saitama 350-0290.



Compounds	R_1	R_2
Docetaxel	$-OH$	$-OC(CH_3)_3$
Paclitaxel	$-OCOCH_3$	$-C_6H_5$

Fig. 1. Chemical structures of docetaxel and paclitaxel.

inhibitory effects of MDR modulators, i.e., cyclosporin A and SDZ PSC 833 (PSC833). Based on these findings, we predicted the clinical effectiveness of MDR modulators for cancer chemotherapy.[14] Here, the interaction of docetaxel with human P-gp was examined by using LLC-GA5-COL150 cells.

MATERIALS AND METHODS

Chemicals [^{14}C]Docetaxel (1.75 GBq/mmol) was a kind gift from Rhône-Poulenc Rorer (Vitry sur Seine, France). [^{3}H]Inulin (38.5 GBq/mmol) was obtained from Amersham International, plc (Buckinghamshire, UK). Cyclosporin A and PSC833 were kindly supplied by Novartis Pharma (Basel, Switzerland). Verapamil hydrochloride, daunorubicin hydrochloride and colchicine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). All other chemicals were of the highest purity available.

Cells and cell culture LLC-PK$_1$ and LLC-GA5-COL150 cells transfected with human *MDR1* cDNA were used. The expression of P-gp on the apical membrane of LLC-GA5-COL150 cells was confirmed previously by immunostaining and electron microscopic immunocytochemistry using the monoclonal antibody MRK16.[11, 12] Both of these cell lines were maintained in complete medium consisting of Medium199 (Dainippon Pharmaceutical Co., Ltd., Osaka) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD) without antibiotics. Monolayer cultures were grown in an atmosphere of 5% CO_2-95% air at 37°C, and were subcultured every 4 and 7 days for LLC-PK$_1$ and LLC-GA5-COL150 cells, respectively, with 0.02% EDTA and 0.05% trypsin (Gibco BRL, Life Technologies, Inc., Grand Island, NY).

Transcellular transport and intracellular accumulation of [^{14}C]docetaxel LLC-PK$_1$ and LLC-GA5-COL150 cells were seeded on microporous polycarbonate membrane filters ("Transwell" 3414, Costar, Cambridge, MA) at a cell density of 4×10^5 and 5×10^5 cells/cm^2, respectively. Cells were cultured in 2.6 and 1.5 ml of Medium199 supplemented with 10% fetal bovine serum and 150 ng/ml colchicine for LLC-GA5-COL150 cells outside and inside the chamber, respectively, in an atmosphere of 5% CO_2-95% air at 37°C for 3 days. The experiments were performed using the same procedure as described previously.[11–15] The transcellular transport assay system using LLC-PK$_1$ and LLC-GA5-COL150 cells is shown schematically in Fig. 2. At 3 h before transport

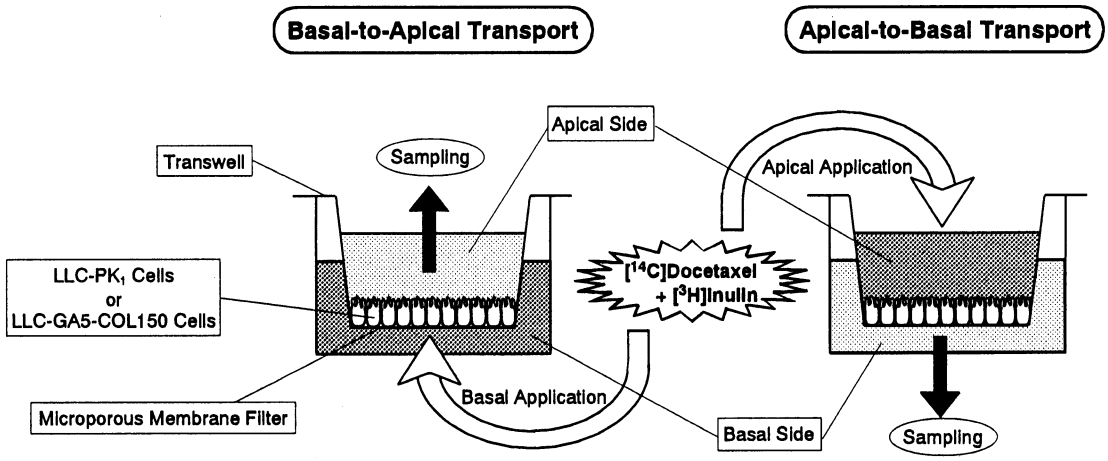


Fig. 2. Scheme of the transcellular transport assay system using LLC-PK$_1$ and LLC-GA5-COL150 cells.

experiments, all culture media were replaced with fresh medium without colchicine. Medium of the donor side was replaced with 2 ml of fresh medium containing the indicated concentrations of [^{14}C]docetaxel (3.4 kBq) and [^{3}H]inulin (34 kBq), and that on the receiver side was replaced with 2 ml of fresh medium alone. In the experiments to examine the time course and temperature dependency of [^{14}C]docetaxel transport, docetaxel was used at 10 μM to saturate its directional transport in LLC-PK$_1$ cells. The monolayers were incubated at 37°C, and aliquots (25 μl) of the medium on the receiver side were taken at the indicated time points. To examine their effects on the transcellular transport of [^{14}C]docetaxel, MDR modulators were added to both sides of the monolayers 1 h before the experiments. The incubation medium also contained the same concentration of MDR modulators. The paracellular leakage was estimated from the amount of [^{3}H]inulin appearing on the receiver side, and it was less than 1% of the total radioactivity per hour. For accumulation studies, the medium was aspirated off at the end of the incubation period, and the monolayers were rapidly washed twice with ice-cold phosphate-buffered saline on each side. The filters with monolayers were detached from the chambers, and the cells on filters were lysed with 1 ml of 0.3 *N* NaOH. The levels of radioactivity of the collected media and lysed cells were counted in 3 ml of ACS II (Amersham International) by liquid scintillation counting (Beckman, LS6000TA, Fullerton, CA) and are presented as percentages of the total radioactivity.

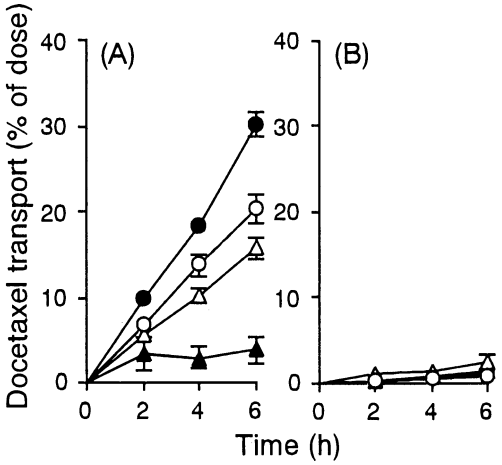


Fig. 3. Transcellular transport of [^{14}C]docetaxel in LLC-PK$_1$ (open symbols) and LLC-GA5-COL150 cells (solid symbols) at 37°C (A) and 4°C (B). The circles (○, ●) indicate the basal-to-apical transport and the triangles (△, ▲) show the apical-to-basal transport. The initial donor concentration of [^{14}C]docetaxel was 10 μM. Each point shows the mean±SE of at least three independent experiments.

RESULTS

Transcellular transport and intracellular accumulation of [^{14}C]docetaxel in LLC-PK$_1$ and LLC-GA5-COL150 cells Fig. 3 shows the transcellular transport of 10 μM [^{14}C]docetaxel in LLC-PK$_1$ and LLC-GA5-COL150 cells at 4°C and 37°C. At 37°C, the basal-to-apical transport of [^{14}C]docetaxel in LLC-GA5-COL150 cells significantly exceeded that in LLC-PK$_1$ cells, whereas the apical-to-basal transport was decreased. The basal-to-apical and apical-to-basal transport of [^{14}C]docetaxel in both cell lines was negligible at 4°C. The intracellular accumulation of [^{14}C]docetaxel after its basal or apical application to LLC-GA5-COL150 cells was 4- to 20-fold lower than that of LLC-PK$_1$ cells at 37°C, also suggesting the overex-

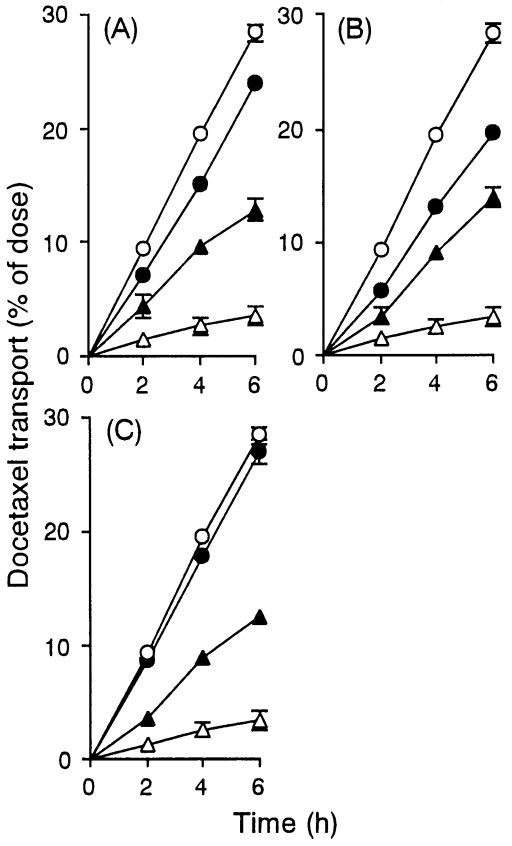


Fig. 4. Effects of cyclosporin A (A), PSC833 (B) and verapamil (C) on the transcellular transport of [^{14}C]docetaxel in LLC-GA5-COL150 cells. Basal-to-apical transport (●) and apical-to-basal transport (▲) in the presence of inhibitors were compared with the same movements in the absence of the inhibitors (○, △). The initial donor concentration of [^{14}C]docetaxel was 0.86 μM, and concentrations of cyclosporin A, PSC833 and verapamil were 20, 5 and 100 μM, respectively. Each point shows the mean±SE of at least three independent experiments.

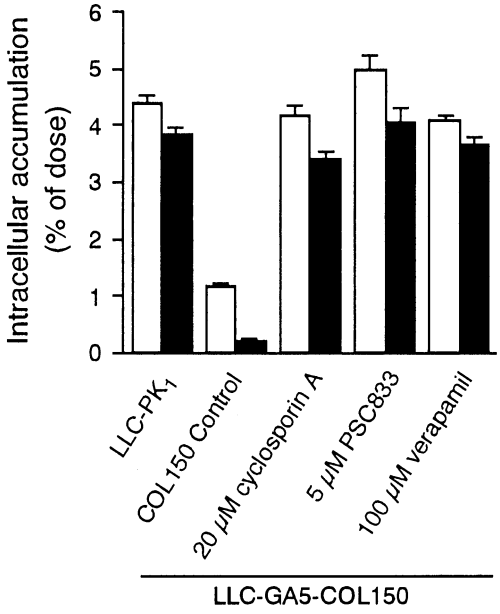


Fig. 5. Intracellular accumulation of [^{14}C]docetaxel in LLC-PK$_1$ and LLC-GA5-COL150 cells. [^{14}C]Docetaxel was added to the basal side (□) or apical side (■). The initial donor concentration of [^{14}C]docetaxel was 0.86 μM. Each column shows the mean±SE of at least three independent experiments.

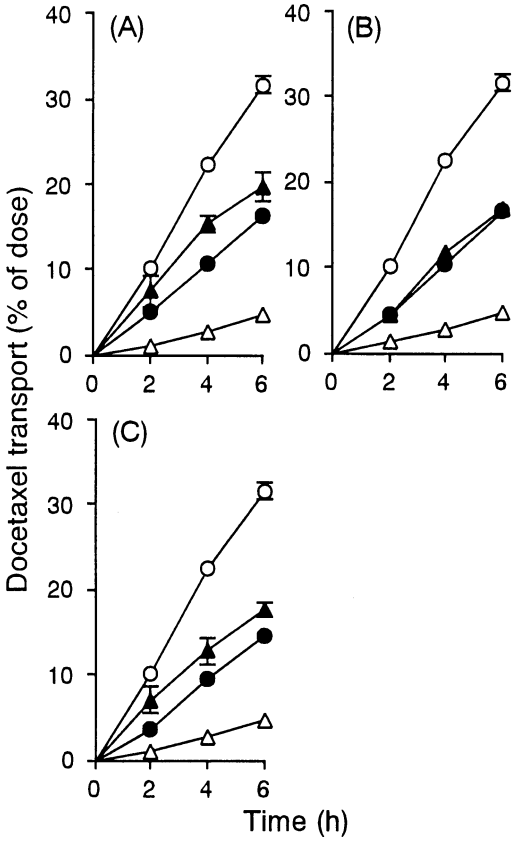


Fig. 6. Effects of cyclosporin A (A), PSC833 (B) and verapamil (C) on the transcellular transport of [^{14}C]docetaxel in LLC-PK$_1$ cells. Basal-to-apical transport (●) and apical-to-basal transport (▲) in the presence of inhibitors were compared with the same movements in the absence of the inhibitors (○, △). The initial donor concentration of [^{14}C]docetaxel was 0.86 μM, and concentrations of cyclosporin A, PSC833 and verapamil were 10, 2 and 10 μM, respectively. Each point shows the mean±SE of at least three independent experiments.

pression of P-gp, resulting in secretion into the extracellular space (Fig. 5).

Inhibitory effects of MDR modulators on the transcellular transport and intracellular accumulation of [^{14}C]docetaxel in LLC-GA5-COL150 cells Fig. 4 shows the inhibitory effects of MDR modulators, i.e., cyclosporin A, PSC833 and verapamil, on the transcellular transport of 0.86 μM [^{14}C]docetaxel in LLC-GA5-COL150 cells. Cyclosporin A (20 μM) and PSC833 (5 μM) inhibited the basal-to-apical transport of [^{14}C]docetaxel, but verapamil (100 μM) had no effect. In contrast, cyclosporin A (20 μM), PSC833 (5 μM) and verapamil (100 μM) all increased the apical-to-basal transport of [^{14}C]docetaxel. They restored the intracellular accumulation of [^{14}C]docetaxel after its basal or apical application to LLC-GA5-COL150 cells to the level seen in LLC-PK$_1$ cells (Fig. 5).

Inhibitory effects of MDR modulators and daunorubicin on the transcellular transport of [^{14}C]docetaxel in LLC-PK$_1$ cells Since LLC-PK$_1$ cells also endogenously expressed P-gp at markedly lower levels than LLC-GA5-COL150 cells,[11, 13, 16, 17] the inhibitory effects of MDR modulators on the transcellular transport of 0.86 μM [^{14}C]docetaxel were also examined using LLC-PK$_1$ cells (Fig. 6). The basal-to-apical transport of [^{14}C]docetaxel was extensively inhibited by cyclosporin A, PSC833 and verapamil at 10, 2 and 10 μM, respectively. These concentrations were 2- to 10-fold lower than those used for LLC-GA5-COL150 cells (Fig. 4). These MDR modulators resulted in an increase in apical-to-basal transport of [^{14}C]docetaxel and consequently in no net transport. The effects of MDR modulators were more marked in LLC-PK$_1$ cells than in LLC-GA5-COL150 cells. Daunorubicin, a substrate for P-gp, also suppressed the [^{14}C]docetaxel transport in a concentration-dependent manner (Fig. 7). Similar results were also obtained with vinblastine (data not shown).

DISCUSSION

Drug-drug interactions may arise as the result of altered pharmacokinetics and pharmacodynamics of the drugs

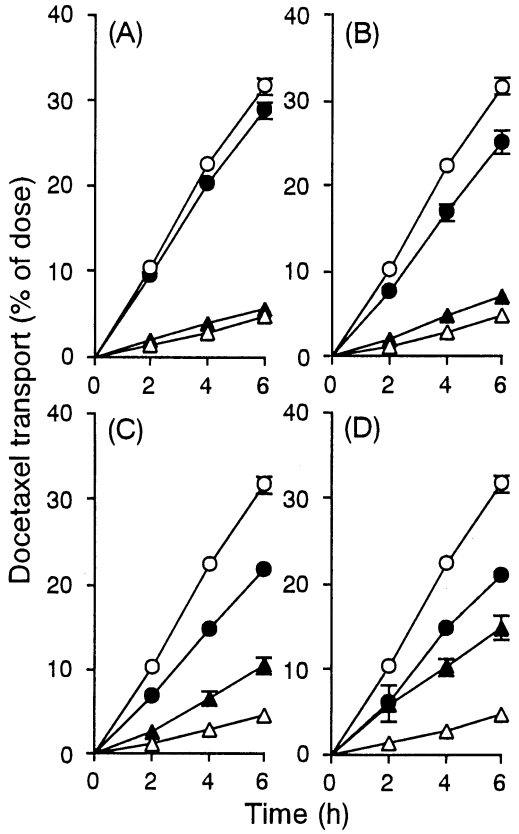


Fig. 7. Effects of daunorubicin on the transcellular transport of [^{14}C]docetaxel in LLC-PK$_1$ cells. Basal-to-apical transport (●) and apical-to-basal transport (▲) in the presence of various concentrations of daunorubicin were compared with the same movements in the absence of daunorubicin (○, △). The initial donor concentration of [^{14}C]docetaxel was 0.86 μM, and concentrations of daunorubicin were 1 μM (A), 3 μM (B), 10 μM (C) and 20 μM (D). Each point shows the mean±SE of at least three independent experiments.

involved. Anticancer drugs are usually given in combination to ensure higher efficacy. Pharmacokinetic interactions are often caused via competitive inhibition of serum proteins, carrier proteins and enzymes. Cytochrome P450 (CYP) 3A4 may be responsible for docetaxel metabolism,[18–20] and docetaxel was shown to be highly bound by plasma proteins (>92%).[21] Therefore, drugs that are substrates of CYP3A4 or that show high binding to plasma proteins should be used with care. However, little is known regarding the carrier system for docetaxel. Recently, P-gp was found to be an important carrier protein located in the biliary canaliculi, the proximal tubules of the kidney, the intestinal and colonic epithelium as well as the capillary endothelial cells of the brain, to have protective effects against noxious xenobiotics and to act as a transporter for endogenous substances.[22, 23] Here, the interaction of docetaxel with P-gp was examined from the viewpoint of pharmacokinetic drug-drug interaction. In addition, P-gp could also be a key protein in pharmacodynamic interactions. P-gp overexpression has been demonstrated to be one of the various mechanisms of MDR in human tumors. P-gp expels anticancer drugs from tumor cells, utilizing the energy produced by hydrolysis of adenosine triphosphate (ATP). Thus, the intracellular concentration of the anticancer drugs is decreased to sublethal levels, resulting in resistance.[23–28] This study was also performed to obtain information regarding the pharmacodynamic drug-drug interactions of docetaxel.

The basal-to-apical transport of [^{14}C]docetaxel in LLC-GA5-COL150 cells significantly exceeded that in LLC-PK$_1$ cells, whereas the apical-to-basal transport was decreased in LLC-GA5-COL150 cells. This can be explained by expulsion of [^{14}C]docetaxel by P-gp overexpressed on the apical membrane of LLC-GA5-COL150 cells.[11, 12] This phenomenon was accompanied by a 4- to 20-fold decrease in the intracellular accumulation of [^{14}C]docetaxel after basal or apical application to LLC-GA5-COL150 cells compared with LLC-PK$_1$ cells. MDR modulators, i.e., cyclosporin A and PSC833, inhibited the basal-to-apical transport and increased the apical-to-basal transport of [^{14}C]docetaxel in LLC-GA5-COL150 cells, whereas verapamil affected only apical-to-basal transport. These changes were also accompanied with increases in the intracellular accumulation of [^{14}C]docetaxel by these three MDR modulators after basal or apical application to LLC-GA5-COL150 cells. We demonstrated previously that cyclosporin A inhibited the P-gp-mediated transport of vinblastine and doxorubicin in LLC-GA5-COL150 cells,[14] corresponding to the increases in plasma concentration or toxicity of vinblastine[29] and doxorubicin[30–32] upon co-administration of cyclosporin A in humans. Consequently, docetaxel was demonstrated to be transported by human P-gp, like vinblastine and doxorubicin. It was expected that the co-administration of MDR modulators, such as cyclosporin A, would increase the plasma concentration of docetaxel in humans.

The observation that verapamil had effects different from those of cyclosporin A and PSC833 on the basal-to-apical transport of [^{14}C]docetaxel in the LLC-GA5-COL150 cells may be explained by the differences in the inhibitory effects of these MDR modulators on P-gp. The present findings are similar to the previous report that itraconazole, an MDR modulator, has a slight effect on the basal-to-apical transport of [^{3}H]vinblastine in LLC-GA5-COL150 cells.[15] These phenomena can be explained by the increased non-P-gp-mediated transport from the intracellular space out of the cells following the increase in intracellular concentration of [^{14}C]docetaxel via inhibition of P-gp.

The inhibition of [^{14}C]docetaxel transport by the MDR modulators, as well as daunorubicin and vinblastine (data not shown) was also found in LLC-PK$_1$ cells. The concentrations of MDR modulators required to provide similar inhibitory effects were lower than those in LLC-GA5-COL150 cells. These observations suggested that pharmacokinetic and pharmacodynamic interactions via P-gp might occur during combination chemotherapy including docetaxel and anticancer drugs, which are substrates of P-gp, since anthracyclines and *vinca* alkaloids have been used in combination chemotherapy with docetaxel.[2)]

In conclusion, our results demonstrated that docetaxel is transported via human P-gp. These observations suggest that pharmacokinetic and pharmacodynamic interactions via P-gp may occur in combination chemotherapy with docetaxel.

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