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ORIGINAL ARTICLE

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P-glycoprotein inhibition by the multidrug resistance-reversing agent MS-209 enhances bioavailability and antitumor efficacy of orally administered paclitaxel

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Abstract *Purpose:* Recent studies in humans and mice have demonstrated that intestinal P-glycoprotein plays a causative role in the limited absorption of orally administered paclitaxel. Multidrug resistance (MDR)-reversing agents, such as cyclosporin A and PSC 833, are known to increase the systemic exposure to orally administered paclitaxel by enhancing absorption in the intestinal tract and decreasing elimination via the biliary tract. In this study, we demonstrated that coadministration of the MDR-reversing agent MS-209, which is known to inhibit P-glycoprotein function by direct interaction, improved the bioavailability of orally administered paclitaxel and consequently enhanced its antitumor activity. *Methods:* The pharmacokinetics of paclitaxel were examined by measuring [^{3}H]paclitaxel in plasma drawn from rats and mice given the drug with or without MS-209. The influence of MS-209 on the intestinal transport of [^{3}H]paclitaxel was studied using a human colorectal cancer cell line, Caco-2. The in vivo efficacy of orally administered paclitaxel in combination with MS-209 was further evaluated in B16 melanoma-bearing mice. *Results:* The plasma concentration of [^{3}H]paclitaxel following oral administration was significantly increased by coadministration of MS-209 at 100 mg/kg in both rats and mice. In rats, the AUC of [^{3}H]paclitaxel following oral administration was strikingly increased (1.9-fold) by coadministration of MS-209, whereas the AUC of [^{3}H]paclitaxel following i.v. injection was slightly increased (1.3-fold) by MS-209. The increase in apparent bioavailability of oral paclitaxel due to MS-209 was 1.4-fold. To demonstrate this enhancing action in vitro, we studied the influence of MS-209 on the transport of [^{3}H]paclitaxel using Caco-2 cells, which is a well-known model of intestinal efflux. The transport of [^{3}H]paclitaxel across the Caco-2 monolayer was markedly inhibited in the presence of MS-209, and the apparent K_i of MS-209 for the active transport of [^{3}H]paclitaxel was 0.4 μM. Moreover, paclitaxel administered orally at 100 mg/kg per day with MS-209 at 100 mg/kg per day showed significant antitumor activity in B16 melanoma-bearing mice, whereas paclitaxel administered orally alone at the same dose showed no antitumor activity. These results suggest that the coadministration of MS-209 improved low systemic exposure to paclitaxel through inhibition of P-glycoprotein, which is involved in drug excretion via the intestinal tract, resulting in a clear antitumor activity of paclitaxel administered orally. *Conclusion:* The present study suggests that coadministration of MS-209 may be a useful way to improve the bioavailability of drugs not suitable for oral administration due to elimination via the intestinal tract.

Keywords P-glycoprotein · Paclitaxel · MS-209 · Oral bioavailability · Antitumor activity

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Introduction

Paclitaxel is one of the drugs indispensable in the treatment of a wide variety of human cancers in the clinic [5, 15]. For clinical use, paclitaxel is currently formulated with Cremophor EL and ethanol (trade name Taxol) and it is administered to patients via i.v. infusion. Oral administration of paclitaxel would offer several advantages over i.v. infusion, e.g. (a) patients would not need to visit the outpatient clinic so frequently, (b) effective plasma levels could be maintained for sufficiently long periods and (c) adverse effects caused by the formulation substance Cremophor EL

would be avoided [24]. However, reports indicating low bioavailability of orally administered paclitaxel in mice have discouraged the effort toward development of an oral formulation [4].

Schinkel et al. [18] generated *mdr1a* gene-deficient mice to study the physiological and pharmacological roles of P-glycoprotein and reported that *mdr1a*(–/–) mice are extremely susceptible to the central neurotoxic pesticide ivermectin, suggesting a protective function of P-glycoprotein against exogenous chemicals in the blood-brain barrier. Subsequent studies using these mice have demonstrated a lower body clearance and a reduced fecal excretion of vinblastine and paclitaxel [25], suggesting that P-glycoprotein also contributes to drug elimination by mediating biliary excretion and/or limiting uptake from the intestinal tract after hepatobiliary excretion of these drugs. Moreover, studies using *mdr1a*(–/–) mice have directly demonstrated that P-glycoprotein strictly limits the uptake from the intestinal tract of paclitaxel administered orally [20], and this has been supported by in vitro experiments [28]. In addition, recent studies in mice and humans have also demonstrated that multidrug resistance (MDR)-reversing agents, such as cyclosporin A and its derivative PSC833, increase the area under the plasma concentration-time curve (AUC) of paclitaxel administered orally [10, 11, 26, 27]. The results of these studies suggest an important role of P-glycoprotein in the elimination of exogenous chemicals, including toxins and anticancer agents, that would restrict the uptake and enhance the efflux of such agents.

MS-209 is an MDR-reversing agent which inhibits the functions of P-glycoprotein [17] and MDR-associated protein (MRP) [14] and has fewer side effects than verapamil, which is a Ca^{2+}-channel blocker known to possess MDR-reversing activity [2, 13, 16, 17]. In the present study, we demonstrated that coadministration of MS-209 markedly improved the bioavailability of oral paclitaxel in rats and mice, and significantly enhanced the antitumor activity of orally administered paclitaxel in a mouse model.

Materials and methods

Drugs

Paclitaxel and [^{3}H]paclitaxel (14.4 Ci/mmol) were purchased from Biolyse (Quebec, Canada) and Moravek Biochemicals (Brea, Calif.), respectively. MS-209 was synthesized by Mitsui Chemicals (Chiba, Japan). Cremophor EL was obtained from BASF Japan (Tokyo, Japan).

Animals

Male SD rats (Japan CLEA, Tokyo, Japan) at 8 weeks of age were used for the pharmacokinetic study. Male ICR mice for the pharmacokinetic study and female CDF1 mice for the antitumor study at 7 weeks of age were purchased from Japan SLC (Shizuoka, Japan).

Pharmacokinetic study

The rats and mice were fasted from 16 h before to 6 h after treatment. [^{3}H]Paclitaxel was dissolved in Cremophor EL and ethanol (1:1, v/v) and diluted fourfold with saline, and the final solution (20 μCi/2.34 μmol/ml) was administered orally or i.v. to rats (4 mg/kg) or mice (8 mg/kg). MS-209 was suspended in water containing 0.1% Tween 80 and was given orally at 100 mg/kg 30 min before administration of [^{3}H]paclitaxel. Blood samples were collected from rats (four animals per group) into heparinized tubes from the jugular vein using heparinized syringes at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 9 and 24 h after administration of [^{3}H]paclitaxel. Blood samples were obtained from mice (four animals per group) at 1 and 2 h after administration of [^{3}H]paclitaxel by cardiac puncture under diethylether anesthesia and collected into heparinized tubes. The plasma fractions were separated by centrifugation (4500 *g*, 3 min), and the [^{3}H]paclitaxel concentrations in plasma were determined using a liquid scintillation counter based on the level of radioactivity. In the rat experiments, the AUC of [^{3}H]paclitaxel up to 24 h was calculated by the trapezoidal rule, and the *t*-test was used for statistical evaluation. The apparent bioavailability (F) of orally administered drug was calculated from the following equation:

$$F(\%) = AUC_{oral}/AUC_{i.v.} \times 100$$

Intestinal efflux model

A human colon adenocarcinoma cell line, Caco-2, was grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 1% non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin in humidified air containing 5% CO_2 at 37°C. The cells were seeded at a density of 1×10^5 cells per permeable polycarbonate insert of the transwell (1.0 cm diameter, 3.0 μm pore size) of 12-well plates (Corning Costar Corporation, Cambridge, Mass.), and the culture medium was changed every 2 days until the experiment. Immediately before the experiment, the cell layers were washed with Hanks' balanced salt solution containing 25 m*M* HEPES, pH 7.4 (HBSS/HEPES). The buffer in the donor (well bottom) side of the cell layer was then replaced with HBSS/HEPES buffer containing [^{3}H]paclitaxel together with MS-209 at 0, 3 or 6 μ*M*, and the buffer in the receiver (insert) side was replaced with HBSS/HEPES buffer containing unlabeled paclitaxel and MS-209 at the same concentrations used in the donor side. The apical (insert) side of the cell layer contained 0.5 ml, and the basal (well bottom) side contained 1.5 ml of solution. The paclitaxel solution (1, 3 or 10 μ*M*) contained [^{3}H]paclitaxel (0.2 μCi/ml) and not more than 0.5% dimethylsulfoxide. After the cell layers had been incubated in air containing 5% CO_2 at 37°C for 1 h, a 300-μl aliquot of the incubation medium was taken from the receiver side and the radioactivity was measured to estimate the [^{3}H]paclitaxel transport from the basal side to the apical side. The transport was expressed as the percentage of radioactivity measured in the apical side or the basal side in relation to the amount of labeled paclitaxel initially added to each side. Net basal-to-apical transport was calculated by subtracting apical-to-basal transport from basal-to-apical transport.

In vivo antitumor activity

The mouse melanoma cell line B16 was maintained in vivo as a xenograft implanted subcutaneously (s.c.) in mice of the strain C57BL/6 (Japan SLC). For the antitumor study, a single cell suspension of B16 cells (1×10^6/mouse) prepared by digestion of the xenograft with dispase (Godo Shusei Company, Tokyo, Japan) was injected s.c. into the flank of a CDF1 mouse. Paclitaxel was dissolved in Cremophor EL and ethanol (1:1, v/v). This solution was diluted fourfold with saline and was administered orally or i.v. daily for 5 days starting the day after tumor inoculation.

MS-209 at 100 mg/kg per day, suspended in water containing 0.1% Tween-80, was given orally 30 min before administration of paclitaxel at 50 or 100 mg/kg per day. The length and width of tumors were measured twice a week, and the tumor volume was calculated with the following equation:

$$\text{Tumor volume } (mm^3) = ([\text{length } (mm)] \times [\text{width } (mm)]^2)/2$$

Seven or nine mice were used per group and the mice were allowed free access to food and water. Dunnett's test was used for statistical evaluation.

Results

Effect of MS-209 on the bioavailability of orally administered [^{3}H]paclitaxel

Coadministration of MS-209 with [^{3}H]paclitaxel resulted in a significant increase in the plasma concentration of [^{3}H]paclitaxel administered orally in both rats and mice. In rats, although the coadministration of MS-209 at 100 mg/kg resulted in a 1.3-fold higher AUC of [^{3}H]paclitaxel injected i.v., the AUC of [^{3}H]paclitaxel administered orally at the same dose was increased 1.9-fold in the presence of MS-209 (Fig. 1 and Table 1). The maximum plasma concentration (C_{max}) of [^{3}H]paclitaxel administered orally in combination with MS-209 was 2.5-fold higher than that of [^{3}H]paclitaxel administered alone, and the apparent bioavailability (F) of orally administered paclitaxel when combined with MS-209 was 1.4-fold higher than that of paclitaxel when administered alone (Fig. 1 and Table 1). These results suggest that the increase in the AUC of [^{3}H]paclitaxel administered orally induced by MS-209 could mainly be attributed to enhanced absorption of paclitaxel in the gastrointestinal tract, and not to a diminished elimination via the biliary tract. The same effect of MS-209 was also observed in mice, in which treatment with MS-209 resulted in 3.7-fold and 4.5-fold higher plasma concentrations of [^{3}H]paclitaxel administered orally compared to the plasma concentrations following treatment without MS-209 at 1 h and 2 h, respectively (Fig. 2).

Effect of MS-209 on [^{3}H]paclitaxel transport in Caco-2 cells

Paclitaxel is known to be a substrate of P-glycoprotein and is transported across a Caco-2 cell monolayer, and this is widely used as a standard model for intestinal efflux, since this cell line exhibits typical features of intestinal epithelial cells [29]. We therefore used this in vitro model to determine whether MS-209 inhibited the intestinal efflux and consequently enhanced the absorption of paclitaxel. MS-209 inhibited [^{3}H]paclitaxel transport across the Caco-2 cell monolayer from the basal side to the apical side in a concentration-dependent manner. The [^{3}H]paclitaxel transport was almost completely inhibited in the presence of 6 μM MS-209

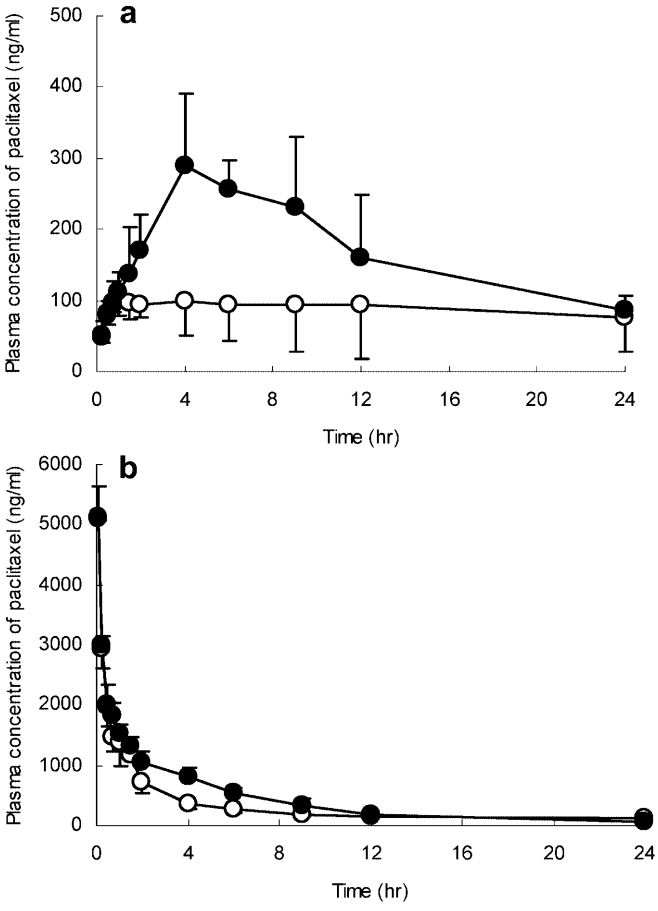

Fig. 1A, B Plasma concentration of paclitaxel in rats after oral (**A**) and i.v. (**B**) administration of [^{3}H]paclitaxel (4 mg/kg) alone or 30 min after oral administration of MS-209 (100 mg/kg) (*filled circles* concentration after administration with MS-209, *open circles* concentration after administration without MS-209)

(Fig. 3A) and a Dixon plot of the inhibition data indicated that MS-209 inhibited [^{3}H]paclitaxel transport with an apparent K_i of 0.4 μM (Fig. 3B). A previous study has indicated that MS-209 inhibits the active transport of antitumor agents by directly interacting with P-glycoprotein on the cancer cell membrane [17]. Our result suggests that MS-209 inhibited the active transport of paclitaxel from the basal side to the apical side in Caco-2 cells in the same manner. Together with the pharmacokinetic data obtained in rats and mice, these results suggest that MS-209 enhanced the absorption of [^{3}H]paclitaxel by inhibiting P-glycoprotein in the intestinal tract.

Enhancement of in vivo antitumor activity of orally administered paclitaxel by MS-209

To further demonstrate the usefulness of MS-209 in improving the bioavailability of orally administered paclitaxel, we evaluated in vivo antitumor activity of paclitaxel administered orally in combination with MS-209. A tumor xenograft of B16 melanoma was used

Table 1 Effect of MS-209 on pharmacokinetic parameters of [^{3}H]paclitaxel at 4 mg/kg after i.v. or oral administration in rats. Data are presented as means ± SD. Statistical significance was assessed by the *t*-test ($AUC_{0-24\ h}$ area under the plasma concentration-time curve up to 24 h, C_{max} maximum plasma level, *Cl* clearance, *F* apparent oral bioavailability)

Parameter	i.v. administration		Oral administration	
	Control	MS-209	Control	MS-209
$AUC_{0-24\ h}$ (mg·h/l)	8.09 ± 0.99	10.84 ± 1.69	2.13 ± 1.25	3.99 ± 0.87*
Cl (l/h per kg)	0.50 ± 0.06	0.38 ± 0.06*	–	–
C_{max} (mg/l)	–	–	0.14 ± 0.05	0.35 ± 0.05*
F (%)	–	–	26.3	36.8

*$P < 0.05$, vs rats administered paclitaxel alone

because the cells show little expression of P-glycoprotein, so tumor growth could be considered to be directly affected by increases in the plasma concentration of paclitaxel as a consequence of the improved bioavailability. Paclitaxel administered orally alone at 50 or 100 mg/kg per day produced no significant inhibition of tumor growth (Fig. 4). However, in combination with MS-209 at 100 mg/kg per day, tumor growth was significantly inhibited by paclitaxel administered orally at 100 mg/kg per day (Fig. 4), and this inhibitory effect was equivalent to that of paclitaxel administered i.v. alone at 20 mg/kg per day (Fig. 4). Interestingly, paclitaxel administered orally in combination with MS-209 caused no toxic deaths and there were no differences in body weight change during the monitoring period in comparison with that following administration of paclitaxel alone. The results of this in vivo antitumor study suggest that inhibition of active efflux in the intestinal tract by MS-209 improved the bioavailability and, thereby, the antitumor activity of orally administered paclitaxel.

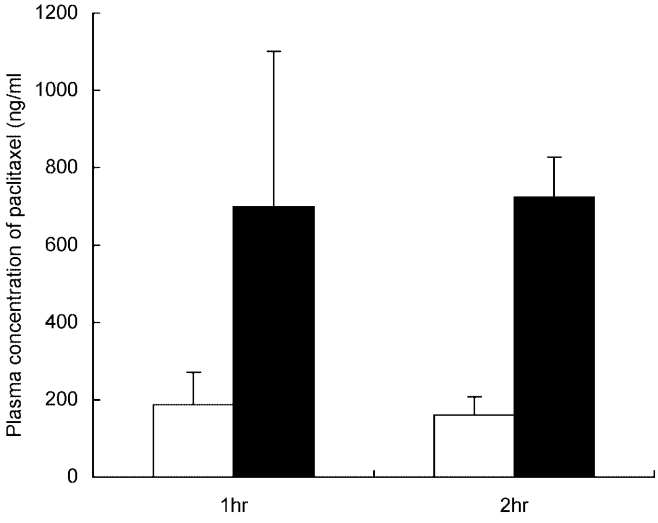

Fig. 2 Plasma concentration of paclitaxel in mice 1 and 2 h after oral administration of [^{3}H]paclitaxel (8 mg/kg) alone or 30 min after oral administration of MS-209 (100 mg/kg) (*filled bars* concentration after administration with MS-209, *open bars* concentration after administration without MS-209)

Discussion

Oral administration is the most preferred and conventional route for drug administration because it is a noninvasive and natural. The overall bioavailability of an orally administered drug depends on many factors, including the morphological and biochemical state of the intestinal epithelium, as well as the physicochemical properties of the drug. Although P-glycoprotein was originally discovered due to its association with acquired drug resistance, it is now recognized as one of the physiological transport systems of a variety of exogenous and endogenous chemicals, such as toxins, antitumor agents and lipids, at particular sites of the body, including the blood-brain barrier, biliary tract and intestinal tract. Paclitaxel is known to be very susceptible to P-glycoprotein-mediated transport, although it has become one of the most important drugs in cancer treatment. It has been reported that the plasma concentration of paclitaxel in mice is very low after oral administration [20].

The bioavailability of orally administered paclitaxel is known to be underestimated because of its nonlinear pharmacokinetics [19]. However, the main factor leading to the low bioavailability of orally administered paclitaxel is considered to be active efflux within the intestinal tract, which is known to express high levels of P-glycoprotein presumably as a secretory detoxifying system [6]. Recent studies have shown that the plasma concentration of paclitaxel is elevated not only in *mdr1a*(–/–) mice but also in normal mice when the drug is administered orally in combination with MDR-reversing agents such as cyclosporin A and its derivative PSC 833 [26, 27]. We therefore expected that, similar to the findings with cyclosporin A, the plasma concentration of paclitaxel in rats and mice would increase if the drug were administered orally in combination with MS-209. In the present study, we did observe a striking increase in the AUC of paclitaxel both in rats and mice when the drug was administered orally in combination with MS-209.

We next demonstrated that MS-209 inhibited the efflux of paclitaxel in Caco-2 cells, a cell line known to possess a differentiated, polarized phenotype with characteristics of absorptive intestinal epithelial enterocytes [1, 7, 8, 12]. The imbalance in the localization of

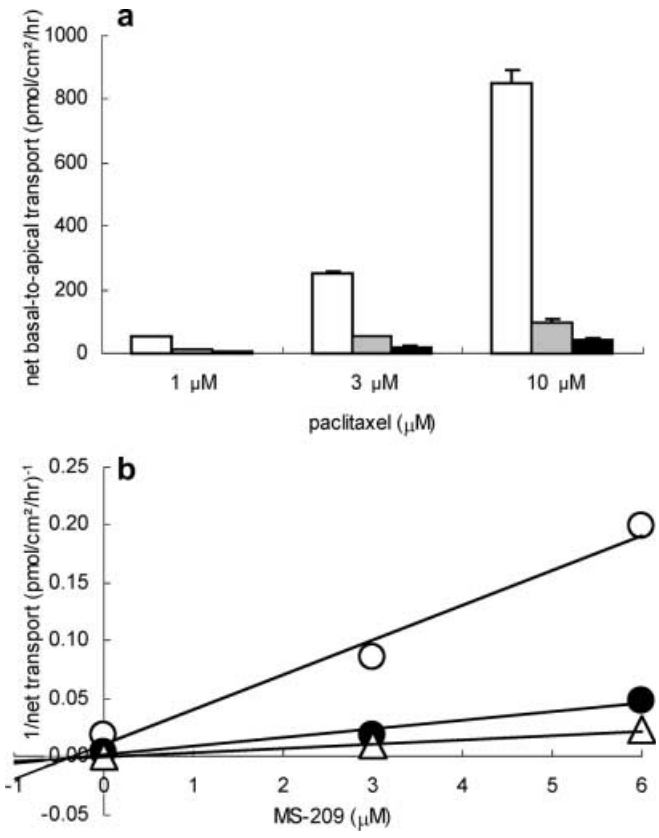

Fig. 3A, B Effect of MS-209 on [^{3}H]paclitaxel transport across Caco-2 cells. **A** Net basal-to-apical transport of 1, 3 or 10 μ*M* [^{3}H]paclitaxel in the absence or presence of various concentrations of MS-209 (*open bars* 0 μ*M*, *cross-hatched bars* 3 μ*M*, *closed bars* 6 μ*M* MS-209). Each bar represents the mean of three wells. **B** Dixon plot of the inhibition of paclitaxel transport across Caco-2 cells (*open circles* 1 μ*M*, *closed circles* 3 μ*M*, *open triangles* 10 μ*M* paclitaxel)

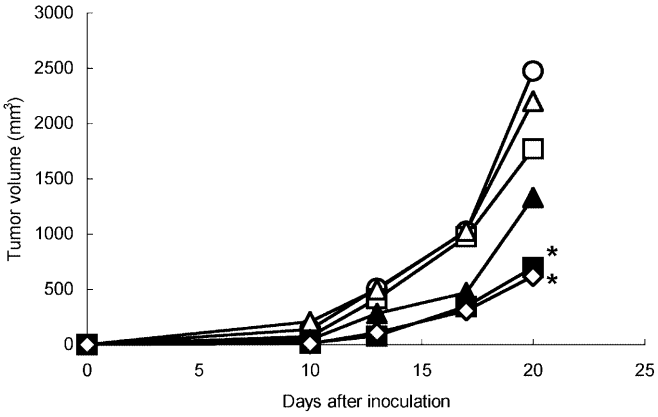

Fig. 4 In vivo efficacy of paclitaxel administered orally in combination with MS-209 in B16 melanoma-bearing mice. B16 melanoma cells (10^6) were implanted subcutaneously (day 0) and the drugs were administered daily for 5 days from day 1. Changes in tumor volume after oral administration of vehicle (*open circles*), after oral administration of paclitaxel at 50 mg/kg per day with (*closed triangles*) or without (*open triangles*) MS-209 at 100 mg/kg per day, after oral administration of paclitaxel at 100 mg/kg per day with (*closed squares*) or without (*open squares*) MS-209 at 100 mg/kg per day, and after i.v. injection of paclitaxel alone at 20 mg/kg per day (*diamonds*) are shown. Each point represents the average tumor volume among nine or seven mice. $*P < 0.05$ vs control, Dunnett's test

P-glycoprotein on the apical membrane of Caco-2 cells is consistent with one of the proposed roles of P-glycoprotein as a secretory detoxifying system and is considered to be directly associated with net basal-to-apical transport of antitumor agents such as paclitaxel and vinblastine in the cells [8, 29]. In addition, several studies have demonstrated a reduction in drug transport after treatment with the P-glycoprotein-specific antibody MRK16 or the MDR-reversing agent verapamil in the Caco-2 model. In the present study, we showed that the net basal-to-apical transport of [^{3}H]paclitaxel was markedly inhibited in the presence of MS-209 at 3 μ*M*, a concentration sufficient to inhibit P-glycoprotein-mediated transport and achievable in mouse and rat plasma by oral administration of MS-209 at 100 mg/kg [17].

As already mentioned, it has been reported that systemic exposure to orally administered paclitaxel is substantially enhanced by coadministration of the MDR-reversing agents cyclosporin A and PSC 833 [26, 27]. Those studies showed that, in normal mice, combination with cyclosporin A or PSC833 enhances the bioavailability of orally administered paclitaxel to levels similar to those obtained in *mdr1a*(–/–) mice given paclitaxel alone. Several in vivo studies have demonstrated that the biliary excretion of P-glycoprotein substrates, including paclitaxel, injected i.v. is inhibited by coadministration of cyclosporin A and PSC833 [3, 21, 22, 23]. However, the biliary excretion of paclitaxel injected i.v. is not significantly different between normal and *mdr1a*(–/–) mice [20], indicating that the main excretion of paclitaxel is not via the biliary tract but via another pathway. It is therefore suggested that the low bioavailability of orally administered paclitaxel in normal mice is not due to biliary excretion but mainly to limited uptake in the intestinal tract, which is mediated by P-glycoprotein.

The AUC of paclitaxel following oral administration with cyclosporin A or PSC833 in normal mice is more than two times higher than that of paclitaxel administered orally alone in *mdr1a*(–/–) mice [20, 26, 27]. In addition, the coadministration of cyclosporin A also increases the AUC of paclitaxel injected i.v. (3.3-fold) in normal mice, which is clearly higher than the ratio (twofold) of the AUC of paclitaxel injected i.v. in *mdr1a*(–/–) to that in normal mice [20, 27]. These studies therefore suggest that cyclosporin A enhances the plasma concentration of paclitaxel following oral administration not only by inhibiting the P-glycoprotein-mediated drug transport but also by modulating other mechanisms involved in drug metabolism such as cytochrome P450 isoenzymes, catalytic enzymes known to be expressed in liver and enterocytes. In fact, cyclosporin A and PSC 833 have been reported to be substrates of cytochrome P450 3A4 isozymes [30]. Furthermore, it has been shown that PSC 833 inhibits the biliary excretion of P-glycoprotein substrates in both normal mice and *mdr1a*/*1b* (–/–) mice [9].

Therefore, these drugs are likely to compete with paclitaxel in the metabolism and biliary excretion in the liver and to inhibit the biliary secretion of paclitaxel. The metabolic competition of these drugs for liver function may imply a potential risk regarding their combination with anticancer agents. In contrast to cyclosporin A and PSC 833, MS-209 seems to show little metabolic competition with paclitaxel because the present study showed that, in rats, the combination of MS-209 resulted in a 1.3-fold higher AUC of paclitaxel when injected i.v. compared to that of paclitaxel administered alone, whereas the increase in AUC was 3.3-fold in the case of the combination of cyclosporin A in mice. Moreover, the apparent bioavailability of orally administered paclitaxel in rats given MS-209 was 36.8%, which is similar to that observed in *mdr1a*(–/–) mice. Thus, the pharmacokinetic data in the present study, similar to the data obtained in *mdr1a*(–/–) mice, indicate that MS-209 increases the plasma level of paclitaxel administered orally mainly by blocking the pump function of P-glycoprotein in the intestinal tract but not through metabolic competition in the liver.

Finally, significant in vivo antitumor activity of paclitaxel administered orally at 100 mg/kg per day in combination with MS-209 at 100 mg/kg per day against B16 melanoma xenografts was demonstrated, whereas paclitaxel administered orally alone showed no significant antitumor activity. P-glycoprotein was scarcely expressed in B16 cells, and MS-209 did not enhance the cytotoxicity of paclitaxel in B16 cells in vitro (data not shown). Therefore, the in vivo efficacy of paclitaxel administered orally in combination with MS-209 was considered to be a direct consequence of the elevated plasma level of paclitaxel through inhibition of P-glycoprotein in the intestinal tract. We consider that this result strongly demonstrates that paclitaxel, which is not suitable for oral administration due to its low bioavailability, can exert a clear antitumor efficacy when combined with MDR-reversing agents.

Therefore, our results suggest that MDR-reversing agents, including MS-209, could serve to improve the bioavailability not only of orally administered paclitaxel but also of other agents with low bioavailability due to their transport by P-glycoprotein after oral administration.

References

1. Artursson P (1990) Epithelial transport of drugs in cell culture. I. A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. J Pharm Sci 79:476
2. Baba M, Nakanishi O, Saito A, Miyama Y, Yano O, Shimada S, Fukazawa N, Naito M, Tsuruo T (1995) Relationship between the multidrug resistant gene expression and multidrug resistance reversing activity of MS-209 in various tumor cells. Cancer Chemother Pharmacol 36:361
3. Burgio DE, Gosland MP, McNamara PJ (1998) Effect of P-glycoprotein modulators on etoposide elimination and central nervous system distribution. J Pharmacol Exp Ther 287:911
4. Eiseman JL, Eddington ND, Leslie J, McAuley C, Sentz DL, Zuhowski M, Kujawa JM, Young D, Egorin MJ (1994) Plasma pharmacokinetics and tissue distribution of paclitaxel in CD2F1 mice. Cancer Chemother Pharmacol 34:465
5. Huizing MT, Misser VH, Pieters RC, ten Bokkel Huinink WW, Veenhof CH, Vermorken JB, Pinedo HM, Beijnen JH (1995) Taxanes: a new class of antitumor agents. Cancer Invest 13:381
6. Hunter J, Hirst BH (1997) Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption. Adv Drug Deliv Rev 25:129
7. Hunter J, Hirst BH, Simmons NL (1993) Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers. Pharm Res 10:743
8. Hunter J, Japson MA, Tsuruo T, Simmons NL, Hirst BH (1993) Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 cells: kinetics of vinblastine secretion and interaction with modulators. J Biol Chem 268:14991
9. Mayer U, Wagenaar E, Dorobek B, Beijnen JH, Borst P, Schinkel AH (1997) Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier P-glycoprotein by oral treatment of mice with PSC833. J Clin Invest 100:2430
10. Meerum Terwogt JM, Beijnen JH, ten Bokkel Huinink WW, Rosing H, Schellens JH (1998) Coadministration of cyclosporin enables oral therapy with paclitaxel. Lancet 352:285
11. Meerum Terwogt JM, Malingre MM, Beijnen JH, ten Bokkel Huinink WW, Rosing H, Koopman FJ, van Tellingen O, Swart M, Schellens JH (1999) Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. Clin Cancer Res 5:3379
12. Meunier V, Bourrie M, Berger Y, Fabre G (1995) The human intestinal epithelial cell line Caco-2: pharmacological and pharmacokinetic applications. Cell Biol Toxicol 11:187
13. Nakanishi O, Baba M, Saito A, Yamashita T, Sato W, Abe H, Fukazawa N, Suzuki T, Sato S, Naito M, Tsuruo T (1997) Potentiation of the antitumor activity by a novel quinoline compound, MS-209, in multidrug-resistant solid tumor cell lines. Oncol Res 9:61
14. Narasaki F, Oka M, Fukuda M, Nakano R, Ikeda K, Takatani H, Terashi K, Soda H, Yano O, Nakamura T, Doyle LA, Tsuruo T, Kohno SA (1997) Novel quinoline derivative, MS-209, overcomes drug resistance of human lung cancer cells expressing the multidrug resistance-associated protein (MRP) gene. Cancer Chemother Pharmacol 40:425
15. Rowinsky EK, Donehower RC (1995) Paclitaxel (Taxol). N Engl J Med 332:1004
16. Sato W, Fukazawa N, Suzuki T, Yusa K, Tsuruo T (1991) Circumvention of multidrug resistance by a newly synthesized quinoline derivative, MS-073. Cancer Res 51:2420
17. Sato W, Fukazawa N, Nakanishi O, Baba M, Suzuki T, Yano O, Naito M, Tsuruo T (1995) Reversal of multidrug resistance by a novel quinoline derivative, MS-209. Cancer Chemother Pharmacol 35:271
18. Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, Te Riele HP (1994) Disruption of the mouse *mdr*1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 77:491
19. Sparreboom A, van Tellingen O, Nooijen WJ, Beijnen JH (1996) Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112
20. Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DKF, Borst P, Nooijen WJ, Beijnen JH, van Tellingen O (1997) Limited oral bioavailability and active epithelial exertion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci U S A 94:2031
21. Speeg KV, Maldonado AL (1994) Effect of the nonimmunosuppressive analogue SDZ PSC-833 on colchicine and doxorubicin biliary secretion by the rat in vivo. Cancer Chemother Pharmacol 34:133
22. Speeg KV, Maldonado AL, Liaci J, Muirhead D (1992) Effect of cyclosporine and colchicine secretion by a liver canalicular transporter studied in vivo. Hepatology 15:899

23. Speeg KV, Maldonado AL, Liaci J, Muirhead D (1992) Effect of cyclosporine on colchicine secretion by the kidney multidrug transporter studied in vivo. J Pharmacol Exp Ther 261:50
24. Theis JG, Liau-Chu M, Chan HS, Doyle J, Greenberg ML, Koren G (1995) Anaphylactoid reactions in children receiving high-dose intravenous cyclosporin for reversal of tumor resistance: the causative role of improper dissolution of Cremophor EL. J Clin Oncol 13:2508
25. van Asperen J, Schinkel AH, Beijnen JH, Nooijen WJ, Borst P, van Tellingen O (1996) Altered pharmacokinetics of vinblastine in *mdr*1a P-glycoprotein-deficient mice. J Natl Cancer Inst 88:994
26. van Asperen J, van Tellingen O, Sparreboom A, Schinkel AH, Borst P, Nooijen WJ, Beijnen JH (1997) Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. Br J Cancer 76:1181
27. van Asperen J, van Tellingen O, van der Valk MA, Rozenhart M, Beijnen JH (1998) Enhanced oral absorption and decreased elimination of paclitaxel in mice cotreated with cyclosporin A. Clin Cancer Res 4:2293
28. Wacher VJ, Salphati L, Benet LZ (1996) Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv Drug Deliv Rev 20:99
29. Walle UK, Walle T (1997) Taxol transport by human intestinal epithelial Caco-2 cells. Drug Metab Dispos 29:343
30. Wandel C, Kim RB, Kajiji S, Guengerich P, Wilkinson GR, Wood AJ (1999) P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. Cancer Res 59:3944