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Short report

Effect of SDZ PSC 833 ([3′-keto-Bmt[1]]-[Val[2]]-cyclosporin) on serum protein binding and distribution to blood cells of doxorubicin, vincristine and etoposide *in vitro*

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SDZ PSC 833 ([3′-keto-Bmt[1]]-[Val[2]]-cyclosporin) is a P-glycoprotein-mediated multidrug resistance modulator currently undergoing clinical trials. SDZ PSC 833 modulates not only antitumor activity but also tissue distribution of doxorubicin in mice. Since protein binding in plasma/serum and distribution to blood cells are important factors affecting the tissue distribution and excretion of drugs, we investigated the effect of SDZ PSC 833 on serum protein binding and distribution to blood cells of doxorubicin, vincristine and etoposide *in vitro*. Unbound fractions in serum and fractions distributed to blood cells of either [^{14}C]doxorubicin, [^{3}H]vincristine or [^{3}H]etoposide were determined using serum and blood obtained from mice and healthy volunteers. Effects of SDZ PSC 833 at 3 μg/ml, which was an achievable concentration in a clinical trial of SDZ PSC 833, on protein binding and distribution of the drugs to blood cells were negligible in mouse and human blood *in vitro*. The absence of interaction between PSC 833 and the anticancer drugs in protein binding and distribution to blood cells suggested the existence of other mechanisms. Possible interactions are speculated to be inhibition of P-glycoprotein function contributing to drug excretion and tissue distribution and inhibition of drug metabolism mediated by cytochrome P450 3A.

Key words: Multidrug resistance, pharmacokinetic interaction, P-glycoprotein, protein binding, SDZ PSC 833.

Introduction

The development of drug resistance hampers effective cancer chemotherapy. It has been well-established that resistance to diverse drugs, categorized generally as natural products, involves overexpression of the *MDR1* gene encoding the 170 kDa membrane P-glycoprotein, an ATP-driven efflux pump for anthracyclines, vinca alkaloids, taxanes and epipodophyllotoxins.[1–3] One of the strategies to overcome P-glycoprotein-mediated multidrug resistance is combined use of a P-glycoprotein inhibitor such as cyclosporin A and verapamil with the multidrug-resistance-related anticancer drugs.[2,3] Circumvention of multidrug resistance *in vitro* by P-glycoprotein inhibitors is well documented, but their clinical use may be limited by their effects on normal tissues.[3,4] For instance, cardiovascular side effects of verapamil make it difficult to keep its effective plasma concentration.[3,4] In addition, P-glycoprotein inhibitors modulate distribution to some tissues of anticancer drugs such as doxorubicin, etoposide and vincristine *in vivo*.[5] Although the mechanisms involved in the pharmacokinetic interaction have not been fully elucidated, it has been speculated that inhibition by the modulator of P-glycoprotein in the intestines, livers, kidneys and other organs causes increased tissue distribution and/or decreased excretion of the anticancer drugs. Other possible mechanisms for the pharmacokinetic interaction are displacement of protein binding and decreased distribution to bood cells by the modulators. It has been said that such interactions in plasma protein binding and distribution to blood cells modulate tissue distribution and drug excretion.[6–8]

SDZ PSC 833 is a non-immunosuppressive cyclosporin derivative that modulates P-glycoprotein-mediated multidrug resistance *in vitro* and *in vivo*.[9–12] SDZ PSC 833 as well as other modulators modulate the pharmacokinetics of multidrug-resis-

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tance-related drugs.[13–15] SDZ PSC 833 increases the tissue distribution of doxorubicin in mice, and the area under the plasma concentration–time curve of etoposide in rats and patients. One possible mechanism is that interaction of SDZ PSC 833 with protein binding and distribution in blood cells of anticancer drugs modifies the pharmacokinetics. The purpose of this study was to examine the effect of SDZ PSC 833 on serum protein binding and distribution in blood cells of doxorubicin, vincristine and etoposide *in vitro*.

Materials and methods

Materials and animals

SDZ PSC 833, [3′-keto-Bmt[1]-[Val[2]]-cyclosporin, was prepared by Sandoz (Basel, Switzerland). [^{14}C]Doxorubicin hydrochloride with specific activity 3.30–3.37 MBq/mg and [^{3}H]vincristine sulfate with specific activity 303–718 MBq/mg were purchased from Amersham (Tokyo, Japan), and [^{3}H]etoposide with specific activity 41.3 MBq/mg was purchased from Moravek Biochemicals (Bre, CA). The purity of the radiochemicals was confirmed to be more than 93.9% using high performance liquid chromatography. CDF1 female mice aged 6 weeks were purchased from Charles River Japan (Atsugi, Japan). They were subjected to examinations at 7 and 10 weeks old.

Determination of unbound fraction in serum

Serum was prepared from blood samples obtained from CDF1 mice and healthy volunteers by centrifugation after standing for 60 min at room temperature, and the resultant supernatant was used in the following experiments. Serum (250 μl) was spiked with a radiolabeled drug with or without SDZ PSC 833 and incubated for 30 min at 37°C to allow equilibration. The final concentrations of the anticancer drugs were 0.37 kbq/ml for [^{14}C]doxorubicin, 3.7 kbq/ml for [^{3}H]vincristine and [^{3}H]etoposide, and 0.3 or 3.0 μg/ml for SDZ PSC 833. These concentrations were relevant to a clinical concentration of SDZ PSC 833 in a phase I study.[15] The unbound fraction of the anticancer drug in the serum was determined by an ultracentrifugation method.[16] In brief, the samples were centrifuged at 15 000 *g* at 25°C for 15 h. The unbound and total concentrations of radiolabeled drugs were determined from radioactivity in the supernatant and the original serum sample, respectively. The unbound fraction (f_u) was calculated from:[17]

$$f_u = C_{sup}/C_{sample}/R$$

where C_{sup} and C_{sample} denote ligand concentrations in the supernatant and sample, respectively. R denotes the recovery rate from the centrifugation tubes determined in the control experiment. Preliminary experiments showed that R for the radiolabeled ligands in the sera from mice and humans was more than 0.96. Therefore, a recovery rate of 1.0 was used in all calculations.

Determination of distribution to blood cells

Bloof (500 μl) was spiked with the radiolabeled drug with or without SDZ PSC 833 and incubated for 30 min at 37°C to allow equilibration. The final concentrations of the radiolabeled drugs and SDZ PSC 833 were the same as in the serum protein binding experiments. After incubation, aliquots of blood were checked for hematocrit levels (Ht) and residual blood was centrifuged to yield plasma. To evaluate total concentration in the blood, 50 μl of 30% hydrogen peroxide solution was added to 100–150 mg of blood decoloration, to which was added 0.5 ml of tissue decomposition solution (Soluene 350; Packard, Tokyo, Japan). The fraction distributed to blood cells (F_b) and blood-to-plasma distribution ratio (R_b) of the anticancer drugs were estimated from:[18]

$$F_b = 1 - C_p/C_B \times (1 - Ht)$$

$$R_b = C_B/C_p$$

where C_B and C_p denote blood concentration and plasma concentration of the radiolabeled ligand, respectively.

Results

The unbound fraction in the serum of the anticancer drugs with or without SDZ PSC 833 was evaluated by the ultracentifugation method. Tracer concentrations of anticancer drugs were used to avoid saturation of serum protein binding of the drugs. On the other hand, the concentrations of SDZ PSC 833 were clinically relevant. The f_u values of [^{14}C]doxorubicin, [^{3}H]vincristine and [^{3}H]etoposide in human and mouse sera are shown in Table 1. Approximately

Table 1. Effect of SDZ PSC 833 on protein binding in serum *in vitro*

Species/ligand	SDZ PSC 833	Unbound fraction in serum (mean ± SE)[a]
Human		
[^{14}C]doxorubicin	—	0.095 ± 0.003
[^{14}C]doxorubicin	0.3 μg/ml	0.095 ± 0.006
[^{14}C]doxorubicin	3 μg/ml	0.102 ± 0.006
[^{3}H]vincristine	—	0.358 ± 0.004
[^{3}H]vincristine	0.3 μg/ml	0.381 ± 0.007*[b]
[^{3}H]vincristine	3 μg/ml	0.373 ± 0.005*
[^{3}H]etoposide	—	0.071 ± 0.003
[^{3}H]etoposide	0.3 μg/ml	0.077 ± 0.001
[^{3}H]etoposide	3 μg/ml	0.081 ± 0.001*
Mouse		
[^{14}C]doxorubicin	—	0.096 ± 0.006
[^{14}C]doxorubicin	3 μg/ml	0.102 ± 0.005
[^{3}H]vincristine	—	0.290 ± 0.002
[^{3}H]vincristine	3 μg/ml	0.299 ± 0.013
[^{3}H]etoposide	—	0.180 ± 0.007
[^{3}H]etoposide	3 μg/ml	0.192 ± 0.003

[a]Unbound fractions denote the mean ± SE of triplicate experiments.
[b]Statistically different from the unbound fraction in the absence of SDZ PSC 833 (unpaired Student's *t*-test: * $p < 0.05$, ** $p < 0.01$).

90% of the doxorubicin and etoposide, and 70% of the vincristine bound to the serum proteins of mouse and human. Although the f_u values of [^{3}H]vincristine and [^{3}H]etoposide with SDZ PSC 833 in human serum were statistically different from their respective controls, SDZ PSC 833 at either 0.3 or 3.0 μg/ml minimally changed the unbound fraction of the three drugs *in vitro*. In addition, a species difference between mouse and human was suggested in the unbound fractions of etoposide and doxorubicin.

Next, we examine the effects of SDZ PSC 833 on the distribution of the anticancer drugs to blood cells *in vitro*. Concentrations of both SDZ PSC 833 and the radiolabeled drugs used were the same as the serum protein binding experiments. Concentrations of doxorubicin, vincristine and etoposide in blood and plasma and Ht of all samples were determined. The F_b and R_b values of the drugs in human and mouse blood are shown in Table 2. F_b values of approximately 0.5–0.6 suggested that the distribution of these anticancer drugs in blood cells is not extensive. The R_b values of the three drugs confirmed that they were not extensively concentrated in the blood cells. The F_b of doxorubicin in human blood was higher than that in mouse blood, but the F_b of vincristine and etoposide were comparable. Although the F_b and R_b values of [^{3}H]etoposide with SDZ PSC 833 in human and mouse sera were statistically different from their respective controls, SDZ PSC 833 minimally influenced the distribution to blood cells of doxorubicin, vincristine and etoposide in mouse and human blood.

In summary, the effects of SDZ PSC 833 at 3 μg/ml, which was an available concentration in a clinical trial of SDZ PSC 833,[15] on the protein binding and distribution to blood cells of all drugs were negligible in mouse and human blood *in vitro*.

Discussion

Recent studies have demonstrated that SDZ PSC 833 improves the antitumor activity of doxorubicin, vincristine and etoposide in tumor-bearing mouse models, but potentiated their toxic effects in mice.[11–14] Enhanced tissue distribution of the drugs was indicated to be responsible for the potentiation of toxicity of the antitumor drugs by SDZ PSC 833 in animals,[13,14] although the mechanisms of the interaction in pharmacokinetics have not been fully elucidated yet. It is easy to speculate that inhibition of the endogenous functions of P-glycoprotein, such as efflux pump in tissues and excretion into bile and urine, by resistance modulators plays a role in the interaction. This hypothesis is supported by recent findings of inhibition of biliary excretion of P-glycoprotein substrate by cyclosporin A and verapa-

Table 2. Effect of SDZ PSC 833 on the distribution of anticancer drugs to blood cells *in vitro*

Species/ligand	SDZ PSC 833	F_b[a] (mean ± SE)	R_b[b] (mean ± SE)
Human			
[^{14}C]doxorubicin	—	0.631 ± 0.015	1.49 ± 0.04
[^{14}C]doxorubicin	0.3 μg/ml	0.644 ± 0.022	1.55 ± 0.07
[^{14}C]doxorubicin	3 μg/ml	0.618 ± 0.011	1.43 ± 0.02
[^{3}H]vincristine	—	0.620 ± 0.011	1.42 ± 0.00
[^{3}H]vincristine	0.3 μg/ml	0.612 ± 0.009	1.42 ± 0.02
[^{3}H]vincristine	3 μg/ml	0.621 ± 0.019	1.44 ± 0.04
[^{3}H]etoposide	—	0.505 ± 0.011	1.08 ± 0.00
[^{3}H]etoposide	0.3 μg/ml	0.516 ± 0.004	1.12 ± 0.01*
[^{3}H]etoposide	3 μg/ml	0.538 ± 0.007*,[c]	1.18 ± 0.01**
Mouse			
[^{14}C]doxorubicin	—	0.476 ± 0.014	1.11 ± 0.02
[^{14}C]doxorubicin	3 μg/ml	0.514 ± 0.017	1.19 ± 0.03
[^{3}H]vincristine	—	0.628 ± 0.014	1.54 ± 0.03
[^{3}H]vincristine	3 μg/ml	0.636 ± 0.006	1.58 ± 0.01
[^{3}H]etoposide	—	0.475 ± 0.002	1.08 ± 0.01
[^{3}H]etoposide	3 μg/ml	0.523 ± 0.012**	1.19 ± 0.02**

Data denote the mean ± SE of triplicate experiments.
[a]Fraction distributed to blood cells.
[b]Blood-to-plasma concentration ratio.
[c]Statistically different from control (unpaired Student's *t*-test: * $p < 0.05$, ** $p < 0.01$).

mil, and of altered tissue distribution of P-glycoprotein substrates in *mdr1a* knock-out mice.[19–22]

In addition to inhibition by the modulators of the function of P-glycoprotein in normal tissues, other interaction mechanisms should be taken into consideration. Theoretical considerations in pharmacokinetics indicate that multiple factors are involved in the pharmacokinetic interaction, e.g. interactions in P-450-mediated metabolism, protein binding in plasma/serum, distribution in blood cells and binding in tissues.[6] When the unbound fraction of anticancer drug in blood is increased by protein binding displacement and/or reduction in the distribution to blood cells by SDZ PSC 833, there is a transient rise in unbound concentration of anticancer drug in plasma. The increase in unbound concentration in plasma could increase the tissue distribution of the anticancer drug.[6,7] Recently it was demonstrated that verapamil and its metabolites displaced the binding of vincristine to α_1-acid glycoprotein in plasma *in vitro*.[23] These findings indicate the possibility that the multidrug resistance modulators modulate the unbound fraction of anticancer drugs in blood.

To elucidate the mechanisms of pharmacokinetic interaction, it is therefore important to investigate the interaction in plasma/serum protein binding and distribution in blood cells. In this study, we examined the effect of SDZ PSC 833 on the protein binding and distribution to blood cells of doxorubicin, vincristine and etoposide *in vitro*. The present results present the important conclusion that SDZ PSC 833 of at least 3 μg/ml did not markedly influence both serum protein binding and distribution to blood cells of doxorubicin, vincristine and etoposide in human and mouse blood. This indicates that the interaction in plasma protein and blood cells can be excluded from mechanisms for the modification by SDZ PSC 833 of the pharmacokinetics of doxorubicin, vincristine and etoposide. Further investigation on the mechanisms of modification by SDZ PSC 833 of the pharmacokinetics of the anticancer drugs is progressing.

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