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SHORT COMMUNICATION

Michel Lemaire
Armin Bruelisauer · Pierrette Guntz · Hitoshi Sato

Dose-dependent brain penetration of SDZ PSC 833, a novel multidrug resistance-reversing cyclosporin, in rats

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Abstract This study quantitatively assessed the brain penetration of a potent P-glycoprotein inhibitor, SDZ PSC 833, and its effect on the blood-brain barrier (BBB) permeability (PS) of an anticancer agent, vincristine. At lower doses of SDZ PSC 833 the brain penetration, defined as the brain-to-blood partition coefficient (Kp), was very low in spite of the high lipophilicity of this compound. At higher doses, however, the brain penetration of SDZ PSC 833 was markedly increased. Since the blood pharmacokinetics of SDZ PSC 833 proved to be linear in the dose range studied, these results demonstrated a dose-dependent brain passage of SDZ PSC 833. The brain passage of cyclosporin A was also found to be dose-dependent. However, the potency of SDZ PSC 833 in inhibiting the efflux mechanism at the BBB was higher than that of cyclosporin A since 10 times higher doses of cyclosporin A were required to obtain the same Kp values recorded for SDZ PSC 833. Moreover, the coadministration of SDZ PSC 833 increased the brain penetration of cyclosporin A, whereas the latter did not modify that of SDZ PSC 833. The increase in SDZ PSC 833 and vincristine PS values observed at high blood levels of SDZ PSC 833 are consistent with the hypothesis of a saturation of the P-glycoprotein pump present at the BBB. The involvement of P-glycoprotein in the brain passage of SDZ PSC 833 could be of great significance for clinical application of the drug in the treatment of brain cancers when it is given in combination with anticancer agents.

M. Lemaire (✉) · A. Bruelisauer · P. Guntz
Drug Metabolism & Pharmacokinetics, Sandoz Pharma Ltd., Basle, Switzerland Phone/Fax: (+ 41)61-3243159/(+ 41)61-3243273

H. Sato
Faculty of Pharmaceutical Sciences, Department of Hospital Pharmacy, Toyama Medical and Pharmaceutical University, Toyama, Japan
(Fax: (+ 81)764-34-5089)

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Abbreviations *BBB* blood-brain barrier · *Kp* brain-to-blood partition coefficient · *PS* permeability-surface area product

Introduction

Despite its highly lipophilic nature, the transport of cyclosporin A through the blood-brain barrier (BBB) remains unexpectedly low [19]. The low permeability of the BBB to cyclosporin A has been explained by the drug's extensive blood binding [17] and by the presence of a threshold of molecular weight above which transport through the BBB does not increase commensurately with lipid solubility [20].

It has also been reported that P-glycoprotein, an active efflux pump of antitumor agents in multidrug-resistant tumor cells, functions in endothelial cell brain capillaries [14, 26]. On the basis of the resistance-modifying activity of cyclosporin A, in vitro and in vivo studies suggested that the low-level passage of cyclosporin A into the brain was due to its active efflux from the BBB by P-glycoprotein [22, 24, 29]. Recently, a nonimmunosuppressive cyclosporin D analog, SDZ PSC 833, has been reported to reverse multidrug resistance (MDR) and to be approximately 10 times more potent than cyclosporin A [9]. Therefore, the objective of the present study was to examine whether SDZ PSC 833 would penetrate the brain to a larger extent than cyclosporin A and the extent to which SDZ PSC 833 would modulate the BBB passage of vincristine, an anticancer agent. For these purposes we used in vivo approaches based on rat-brain concentration measurements after i.v. administration and brain perfusion.

Materials and methods

Drugs

[^{14}C]-SDZ PSC 833 (44 μCi/mg) and [^{3}H]-cyclosporin A (12.2 mCi/mg) were supplied by Sandoz Pharmaceuticals (Basel, Switzerland). [^{14}C]-SDZ PSC 833 was labeled in position 1 of L-valine 2, whereas [^{3}H]-cyclosporin A was labeled in the Abu-β position. [^{3}H]-Vincristine was purchased from Amersham International (Buckinghamshire, England); according to the manufacturer, the specific activity was 10.4 mCi/mg. The radiochemical purity of all isotopes was assessed by high-performance liquid chromatography (HPLC) and was greater than 95%. The solvent, used as a vehicle for administration of SDZ PSC 833 and cyclosporin A, was a 40:10 (v/v) mixture of polyethylene glycol 200 (PEG200) and ethanol. Vincristine was solubilized in 0.9% NaCl.

Animals

Male Wistar rats (250 ± 10 g) were used for all experiments. The animals were fasted overnight prior to i.v. administration of drugs.

Intravenous bolus-injection studies

Brain distribution of SDZ PSC 833 was examined after i.v. administration (2.8 ml/kg) of the labeled compounds into the femoral vein. Each group of rats ($n = 3$) received the following doses: [^{14}C]-SDZ PSC 833 and [^{3}H]-cyclosporin A at 0.1, 0.3, 1, 3, 10, and 30 mg/kg and [^{3}H]-vincristine at 1 mg/kg. Furthermore, the role of SDZ PSC 833 on the brain penetration of cyclosporin A and vincristine was studied using the following drug combinations: [^{3}H]-cyclosporin A (10 mg/kg)-[^{14}C]-SDZ PSC 833 (10 mg/kg), [^{3}H]-cyclosporin A (0.1 mg/kg)-SDZ PSC 833 (10 mg/kg), and [^{3}H]-vincristine (1 mg/kg)-SDZ PSC 833 (10 mg/kg). At 2 h after drug injection the animals were killed by exsanguination while under light anesthesia, after which the brain was removed and radioactivity was counted in blood and brain samples. At this time, SDZ PSC 833, cyclosporin A, and vincristine are present in both blood and brain, essentially as parent drugs [2, 31]. Therefore, the blood and brain concentrations of radioactivity, expressed in nanomolar values, were considered to be representative of parent drug concentrations. The degree of brain penetration was characterized by the brain/blood drug concentration ratio, Kp.

Internal carotid-artery perfusion/capillary depletion

Blood-brain transfer was also measured in rats anesthetized with a ketamine-xylazine dose using the internal carotid-artery perfusion method [25] followed by capillary depletion [28]. The external carotid artery was cannulated and all branches (i.e., the pterygopalatine, occipital, and superior thyroidal arteries) were closed by electrocoagulation. At time zero, each rat received an i.v. dose of 10 mg SDZ PSC 833; at 5 min or 2 h after injection the brain perfusion was performed. For this purpose, the common carotid artery was ligated and perfusion fluid was infused into the external carotid artery. The perfusate consisted of 0.12 *M* NaCl, 4.7 m*M* KCl, 25 m*M* $NaHCO_3$, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 2.5 m*M* $CaCl_2$, 10 m*M* D-glucose, 0.1 g bovine albumin/ml, and 5 and 1 μCi [^{3}H]-vincristine and [^{14}C]-sucrose or [^{3}H]-sucrose and [^{14}C]-SDZ PSC 833/ml, respectively. Labeled sucrose was used as an internal reference compound for blood-volume determinations in individual animal brains and as a marker for leakage of vascular contents into the brain supernatant during the capillary depletion process. The perfusion fluid was prepared freshly before use to minimize drug breakdown. The solutions were filtered, oxygenated with 95% O_2/5% CO_2, warmed to 37°C, and then perfused with a peristaltic pump (Minipuls3 Gilson, Villiers le Bel, France) at a flow rate of 4.5 ml/min for 20 s. Because there was a 5-s delay during which the pump reached its maximal flow rate, the effective transport time was 15 s.

After the perfusion, each animal was decapitated and the ipsilateral brain was removed. The choroid plexus was carefully removed, and the brain was weighed and homogenized in 3.5 ml physiologic buffer (pH 7.4; 10 m*M* HEPES, 141 m*M* NaCl, 4 m*M* KCl, 2.8 m*M* $CaCl_2$, 1 m*M* $MgSO_4$, 1 m*M* NaH_2PO_4, and 10 m*M* D-glucose). Because the drug could be sequestered by the brain microvasculature with no transfer across the endothelial barrier into the brain parenchyma, it was necessary to differentiate between vascular bed binding and BBB passage. Therefore, the brain homogenate was depleted of microvasculature by density centrifugation via addition of 4.5 ml cold 26% dextran solution (79,000 molecular weight) to the homogenate followed by rehomogenization at 4°C; thereafter, the homogenate was centrifuged at 5000 *g* for 20 min at 4°C in a swinging bucket rotor. The supernatant (transcytosed space/interstitial fluid) and pellet (capillary bed/endothelial cells and pericytes) were carefully separated. The homogenate, pellet, supernatant, and perfusate aliquots were solubilized in 2 ml Packard soluene 350 and prepared for [^{3}H]/[^{14}C]-double-isotope liquid scintillation spectrometry. Volumes of distribution (V_d) for [^{14}C]-SDZ PSC 833, [^{3}H]-vincristine, and [^{14}C] or [^{3}H]-sucrose were calculated for the homogenate, pellet, and postvascular supernatant as: V_d (in microliters per gram) = disintegrations per minute per gram of brain/disintegrations per minute per microliter of infusate. Because some intravascular radioactivity leaked into the supernatant after homogenization and rupture of brain blood vessels, we had to subtract the supernatant V_d recorded for [^{14}C]- or [^{3}H]-sucrose from that calculated for the [^{14}C]- or [^{3}H]-labeled drug. The sucrose V_d was also subtracted from the homogenate V_d or from the pellet V_d to correct for the entrained blood volume.

The supernatant V_d was converted into brain extraction values (E) according to the following equation: $E = V_d/perfusion\ time \times F$, where the perfusion time is 15 s and F is the perfusate flow. The latter was found in a previous study [13] to be 2300 μl min^{-1} g^{-1}. The supernatant permeability-surface area product (PS) was calculated using the equation $PS = -F \times ln(1\text{-}E)$. The permeability, P, was obtained using an S value, i.e., a surface area, for the BBB of 100 cm^2/g brain [21].

Results

Dose dependency of SDZ PSC 833 and cyclosporin A brain distribution

In the wide dose range examined, i.e., 0.1–30 mg/kg, the SDZ PSC 833 and cyclosporin A blood levels measured at 2 h after injection showed a good dose proportionality (Table 1). Due to this linear dose-blood level relationship, the brain penetration of both compounds was characterized by the brain/blood concentration ratio, Kp (Fig. 1). The values corresponding to SDZ PSC 833 indicated a low Kp of about 0.2 in the dose range of 0.1–1 mg/kg followed by a rapid increase up to 1.4 after a 10-mg/kg dose; the Kp value increased only marginally at a 30-mg/kg dose. A similar dose-dependent brain penetration was observed with cyclosporin A at higher doses; very low Kp values of about 0.1 were found in the dose range of 0.1–10 mg/kg, followed by an increase up to 1.1 at 30 mg/kg.

Table 1 Blood and brain levels of total radioactivity observed at 2 h after i.v. administration of [^{14}C]-SDZ PSC 833 or [^{3}H]-cyclosporin A[a]

Dose (mg/kg)	[^{14}C]-SDZ PSC 833		[^{3}H]-Cyclosporin A	
	Brain	Blood	Brain	Blood
0.1	4 ± 0	19 ± 4	2 ± 0	24 ± 3
0.3	11 ± 1	57 ± 9	6 ± 1	89 ± 3
1	54 ± 12	195 ± 53	23 ± 2	364 ± 5
3	605 ± 136	653 ± 58	127 ± 10	909 ± 40
10	3731 ± 368	2593 ± 77	857 ± 77	3030 ± 77
30	8977 ± 153	5842 ± 359	9894 ± 406	8719 ± 647

[a] Data represent mean values ± SD ($n = 3$), expressed as nM values

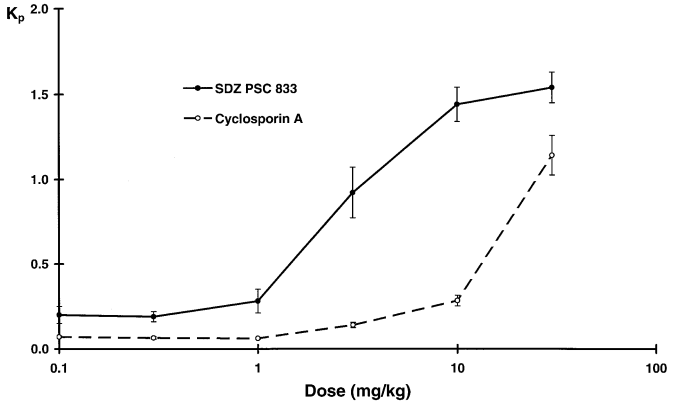

Fig. 1 Brain/blood concentration ratios (Kp) determined for [^{14}C]-SDZ PSC 833 and [^{3}H]-cyclosporin A at 2 h after a single i.v. injection of labeled compound. Data represent mean values ± SD ($n = 3$)

Influence of SDZ PSC 833 on the brain penetration of cyclosporin A and vincristine

As shown in Fig. 2, the coadministration of [^{14}C]-SDZ PSC 833 (10 mg/kg) and [^{3}H]-cyclosporin A (10 mg/kg) resulted in a 5-fold increase in Kp values for cyclosporin A; a similar effect was observed even after the coadministration of 0.1 mg/kg cyclosporin A. In contrast, the Kp value observed for [^{14}C]-SDZ PSC 833 after the coadministration of 10 mg/kg cyclosporin A (Kp = 1.2) was similar to that observed after a single administration of [^{14}C]-SDZ PSC 833. The coadministration of SDZ PSC 833 (10 mg/kg) and [^{3}H]-vincristine (1 mg/kg) resulted in a slight (factor 2) but nonsignificant increase in the vincristine Kp values.

Brain capillary endothelial permeability to SDZ PSC 833

The brain V_d and BBB PS values measured for SDZ PSC 833 by brain perfusion and capillary depletion are given in Table 2. No passage of SDZ PSC 833 was

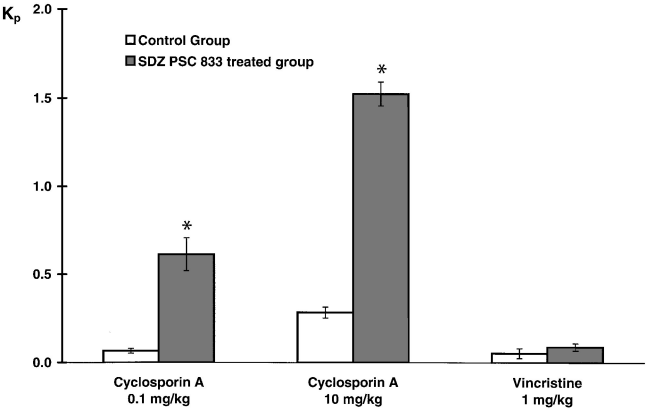

Fig. 2 Effect of SDZ PSC 833 on the brain/blood concentration ratios (Kp) of [^{3}H]-cyclosporin A and [^{3}H]-vincristine. The brain and blood concentrations were measured at 2 h after i.v. injection of the labeled-drug dose alone (Control Group) or in combination with SDZ PSC 833 (10 mg/kg). Data represent mean values ± SD ($n = 3$) *$P < 0.05$ versus the control value (Student's t-test for unpaired data)

detected at 5 min after the injection of 500 µl saline; however, injection of the vehicle for SDZ PSC 833, i.e., a mixture of polyethylene glycol 200 (PEG200) and ethanol, resulted in a significant PS value of 0.029 ml min^{-1} g^{-1}. This permeability of the BBB to SDZ PSC 833 was increased by a factor of 3 (PS = 0.097 ml min^{-1} g^{-1}) when SDZ PSC 833 had previously been injected at a dose of 10 mg/kg. However, at 2 h after i.v. injection the PS product fell again to an undetectable value. The pellet V_d values recorded for SDZ PSC 833 were higher than those obtained with [^{3}H]-sucrose, suggesting that SDZ PSC 833 was sequestered by the brain microvasculature. Nevertheless, at 5 min after i.v. injection of SDZ PSC 833, this sequestration by microvessels was very small in comparison with the brain parenchymal uptake of the drug.

Table 2 also clearly indicates that the injection of 10 mg/kg SDZ PSC 833 significantly enhanced the passage of [^{3}H]-vincristine (PS 0.010 ml min^{-1} g^{-1}). The pellet V_d values (0.5–0.6 µl/g) showed a substantial sequestration of vincristine by the microvascular endothelium; this sequestration represented 20% of the total brain uptake of vincristine after coadministration of SDZ PSC 833.

Discussion

This study was designed to assess the brain penetration of a potent P-glycoprotein inhibitor, SDZ PSC 833, and its effect on the BBB permeability of an anticancer agent, vincristine. In vivo results recorded after i.v. bolus administration confirm the extremely low degree of brain penetration of cyclosporin A in spite of its high lipophilicity [1, 3, 17]. The entry of SDZ PSC 833 into

Table 2 Brain V_d[a] and PS values calculated for [^{14}C]-SDZ PSC 833 and [^{3}H]-vincristine after a 10-mg/kg i.v. injection of SDZ PSC 833 (*NM* not measurable)

Brain perfusion	Dose of SDZ PSC 833 (mg/kg)	Nature of injectate	Time between i.v. dose and brain perfusion (min)	V_d homogenate (μl/g)	V_d supernatant (μl/g)	V_d pellet (μl/g)	PS supernatant (ml min^{-1} g^{-1})
[^{14}C]-SDZ PSC 833	0	Nacl 0.9%	5	2.6 ± 3.3	− 2.2 ± 2.1	2.3 ± 0.2	NM
	0	PEG200/ethanol	5	9.9 ± 1.8	7.3 ± 3.5	2.6 ± 1.0	0.029
	10	PEG200/ethanol	5	31.0 ± 13.1*	23.7 ± 10.7*	2.3 ± 1.1	0.097
	10	PEG200/ethanol	120	1.5 ± 3.1	− 0.8 ± 2.9	0.3 ± 0.3	NM
[^{3}H]-Vincristine	0	PEG200/ethanol	5	− 0.4 ± 0.8	− 0.9 ± 0.8	0.5 ± 0.1	NM
	10	PEG200/ethanol	5	2.6 ± 1.6	2.5 ± 1.2*	0.6 ± 0.4	0.010

*$P < 0.01$ versus the control value (Student's *t*-test for unpaired data)
[a] Data represent mean values ± SD ($n = 4$–6)

the brain was also shown to be restricted by the BBB at low doses, where its blood levels varied between 0.02 and 0.2 μ*M* (Table 1), essentially reflecting a sequestration within the brain microvasculature. This uptake by the brain microvessel endothelial cells was also observed for cyclosporin A [21] and represented more than 50% of the amount of drug crossing the lumenal endothelial plasma membrane. At higher doses, however, the brain penetration of SDZ PSC 833 was markedly increased. On the other hand, linear blood kinetics were observed over the whole dose range. Since the unbound fraction of SDZ PSC 833 in blood was found to be approximately 50% for all the blood concentrations determined (unpublished data, Sandoz), our in vivo approaches clearly demonstrated a dose-dependent brain passage of SDZ PSC 833 under physiologic conditions. The brain penetration of cyclosporin A was also found to be dose-dependent (Fig. 1), indicating that the penetration of cyclosporin A into the brain increases at higher doses. These findings are of particular interest for the use of these cyclosporins in the clinical setting, since their neurotoxicity may appear unexpectedly at higher doses.

To obtain a similar degree of brain penetration, the i.v. dose of cyclosporin A has to be roughly 10 times higher than the SDZ PSC 833 dose (Fig. 1). This difference in the brain distribution of these two cyclosporins may be explained by the different unbound fractions observed in rat blood, i.e., 3% for cyclosporin A [16] and 50% for SDZ PSC 833. The higher lipophilicity of SDZ PSC 833 as compared with cyclosporin A could also explain this discrepancy in brain penetration. The role of lipophilicity on a saturable efflux system in the BBB has recently been suggested with a series of model peptides [4]; thus, the higher lipophilicity of SDZ PSC 833 could also explain its higher degree of BBB passage. Finally, this difference in brain penetration may result from the higher affinity of SDZ PSC 833 for the P-glycoprotein, which is expressed by brain capillary endothelial cells in humans [6, 8, 27, 30], rats [11, 27], and mice [7]. SDZ PSC 833 may have a higher in vivo potency than cyclosporin A in blocking the P-glycoprotein pump at the BBB as judged from our observations that lower doses of SDZ PSC 833 were required to attain the same Kp value and from the finding that the coadministration of SDZ PSC 833 increased the brain distribution of cyclosporin A, whereas cyclosporin A did not influence that of SDZ PSC 833. If this applies to tumor cells, SDZ PSC 833 will have a stronger multidrug resistance (MDR)-reversing activity than cyclosporin A under in vivo conditions.

The increase observed in Kp and PS values for SDZ PSC 833 at higher doses, as well as the marked enhancement of the PS value for vincristine (Table 2), are consistent with the hypothesis of a saturation of the P-glycoprotein pump present in the brain capillary endothelial cells. On the basis of this hypothesis, the blood levels of SDZ PSC 833 observed at short times after i.v. administration, i.e., 16 μ*M* at 5 min postdosing (unpublished data, Sandoz), should be sufficient to inhibit the P-glycoprotein pump present at the BBB, whereas at 2 h postdosing the blood levels of SDZ PSC 833, i.e., 3 μ*M* (Table 1), should be insufficient to do so. These low SDZ PSC 833 blood levels are similar to the vincristine blood levels observed at 2 h after i.v. administration of 1 mg/kg [32] and are consistent with the low Kp values (0.1–0.2) recorded for both compounds. Moreover, similar Kp values observed for SDZ PSC 833 after i.v. doses of 10 and 30 mg/kg (Fig. 1) indicate that in this dose range the brain passage of SDZ PSC 833 obeys a passive diffusion process. These results are consistent with previous in vitro [24, 29] and in vivo [22] studies, which suggested a functional role of P-glycoprotein in actively effluxing cyclosporin A from the brain to the blood.

The present in vivo study clearly demonstrates that the passage of vincristine into the brain is significantly enhanced by SDZ PSC 833. This finding is in accordance with previous in vivo [22] and in vitro [24, 29] studies showing that cyclosporin A increases the brain penetration of vincristine. Of genetic importance to the role of P-glycoprotein in the brain penetration of cytostatic agents is a recent finding that a homozygous disruption of the mouse *mdr1a* P-glycoprotein gene

leads to a deficiency in the BBB [23]. The cerebrovascular permeability, P, of vincristine found in this study amounted to 2×10^{-6} cm/s as measured after SDZ PSC 833 predosing; this permeability value is significantly higher than those of 0.64×10^{-6} [18] and 1.17×10^{-6} cm/s [12] found in untreated rats. This P value observed in SDZ PSC 833-treated animals is nonetheless 50 times lower than that (10^{-4} cm/s) predicted for vincristine from its log P value of 2.80 [18]. Thus, the unexpectedly low permeability of the BBB to vincristine after pretreatment with SDZ PSC 833 may not be explained solely by efflux of the drug by P-glycoprotein.

Instead, it is reasonable to think that the poor brain penetration of vincristine may also be due to the intrinsically low permeability of the BBB to this drug and to its extensive distribution to the capillary endothelium. Thus, the low BBB permeability of vincristine despite its high lipophilicity may be better explained by the combined effects of physical properties of the BBB, capillary uptake, and the efflux function of P-glycoprotein. This concept was recently proposed by Shirai et al. [24], who suggested that the BBB remains an effective barrier to vincristine even when the P-glycoprotein efflux pump does not work. Given this hypothesis, it is noteworthy that cyclosporin A did not modify the brain transport of doxorubicin, another antineoplastic agent, after coadministration of these two compounds to mice [5]. However, the interaction between SDZ PSC 833 and vincristine observed in this study may also be related to other pharmacokinetic interactions observed between SDZ PSC 833 and etoposide [15] or doxorubicin [10]. In the latter studies, increased blood and tissue concentrations of the anticancer drugs may also be related to an inhibition of the P-glycoprotein pump by SDZ PSC 833.

In summary, the present study shows that SDZ PSC 833 governs its own brain penetration in a dose-dependent manner. Moreover, the BBB transport of cyclosporin A and vincristine, both of which are typical substrates of P-glycoprotein, was significantly enhanced by pretreatment with SDZ PSC 833. Therefore, our study shows that P-glycoprotein at the BBB is concerned, at least in part, with the brain penetration of SDZ PSC 833. This involvement of P-glycoprotein in the brain passage of SDZ PSC 833 is of great significance for clinical application of the drug, in terms of both advantageous (i.e., treatment of brain cancers) and disadvantageous (i.e., adverse CNS effects) aspects, when it is given in combination with anticancer drugs.

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