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Short communication

In vivo evidence of complete circumvention of vincristine resistance by a new triazinoaminopiperidine derivative S 9788 in P388/VCR leukemia model

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Summary. S 9788, a new triazinoaminopiperidine derivative, was found to be a potent reversant of vincristine resistance in the in vivo murine leukemic P388/VCR model. In two treatment regimens (Q4D days 1, 5 and 9 and QD days 1–9), S 9788 enhanced the antitumor activity of vincristine in a dose-dependent manner, resulting in a complete circumvention of drug resistance for well-tolerated doses of S 9788. S 9788 was also effective in enhancing therapeutic effects of vincristine in the treatment of sensitive P388-bearing mice. These results strongly suggest that S 9788 may be a potential candidate for circumvention of multidrug resistance (MDR) in clinical practice.

Introduction

A crucial problem in cancer chemotherapy is the development of drug resistance during treatment. In this respect, a great deal of effort is being devoted to overcoming resistance, based on a knowledge of the different mechanisms involved. One particularly well-documented form of resistance at the cellular level is multidrug resistance (MDR). Tumor cells exhibiting this phenotype show cross resistance to a large number of unrelated cytotoxic drugs, such as vinca alkaloids or anthracyclines [1, 9]. It has been demonstrated that the presence of 170,000 kd plasma membrane protein, which is encoded in humans by the mdr_1 gene, is sufficient to produce MDR in cancer cells [6, 17].

The discovery of a MDR phenotype has led many laboratories to search for compounds that could reverse tumor cell resistance. Tsuruo et al. [15] first reported that the combination of the calcium channel blocker verapamil and the cytotoxic agent vincristine (VCR) overcomes the leukemic P388 cell line resistance to vincristine both in vitro and in vivo. In subsequent studies, numerous compounds, including other calcium channel blockers or calmodulin inhibitors, were found to sensitize resistant tumor cells in vitro to antitumor agents and to enhance the activity of cytotoxic drugs in vivo, leading to partial or complete circumvention of the MDR phenotype [3, 5]. Recently, some of these compounds, such as verapamil or AHC-52, have also been found to potentiate the activity of some anticancer drugs on sensitive experimental tumors in vivo [13, 16]. The mechanisms by which all these pharmacological agents modulate the MDR phenotype have not been fully elucidated. However, it appears from numerous studies that P-glycoprotein could be their principal target [5], the interaction between the chemosensitizer and this protein resulting in an inhibition of the drug efflux function [11].

The structural features required by pharmacological agents to reverse MDR have been defined by structure-activity studies [10, 18]. The presence of two polar planar rings, a tertiary basic nitrogen atom, and hydrophobicity were found to be important features shared by potent modulators of MDR. S 9788 is a novel triazinoaminopiperidine derivative with such structural features, which was identified through in vitro screening for its MDR-reversing activity in combination with actinomycin D, in a highly resistant cell line, DC-3F/AD. The fold reversion of resistance to various MDR-associated cytotoxic agents using a panel of human and murine tumor cell lines was 6–300 times greater with S 9788 (5 μM) than with VRP (5 μM).

In the present study, we have evaluated the ability of S 9788 to potentiate the antitumor activity of vincristine on the murine leukemia P388 and P388/VCR models. We investigated two schedules for S 9788 + vincristine combination therapy, and demonstrated that such treatment could result in complete reversion of vincristine resistance in vivo.

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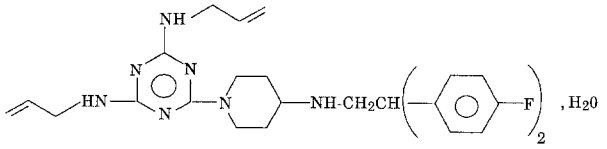

Fig. 1. Chemical structure of the triazinoaminopiperidine derivative S 9788

Materials and methods

Drugs. S 9788 is 6-[4-[2,2-di(4-fluorophenyl)-ethylamino]-1-piperidinyl]-*N*,*N*′-di-2-propenyl-1,3,5-triazine-2,4-diamine (Fig. 1) and was synthesized at the Servier Research Institute (France).

Vincristine sulfate (VCR) was purchased from Roger Bellon (France).

Mice and tumors. Inbred (DBA/2) and hybrid female [CD_2F_1 (BALB/c × DBA/2)] mice weighing 18–22 g were purchased from Charles River (France). They were maintained under specific pathogen-free conditions.

P388 murine leukemia and vincristine-resistant subline P388/VCR were provided by the National Cancer Institute (Bethesda, Md., USA) and were maintained by weekly IP passages in DBA/2 mice. Resistance of P388/VCR cells was retained naturally without any further administration of VCR.

Antitumor activity. S 9788 was suspended in 0.9% NaCl solution containing 0.2% hydroxypropyl cellulose. VCR was dissolved in sterilized water.

CD_2F_1 mice were inoculated IP with 10^6 P388 or P388/VCR cells on day 0. Both drugs were administered IP in a volume of 0.1 ml/10 g body weight, either alone or in combination with VCR, S 9788 being given 60 min before VCR.

Two treatment schedules were evaluated: a total of three administrations, 4 days apart, starting from day 1 (days 1, 5, 9) and one administration daily for 9 days (days 1–9).

Ten mice were used for each experimental group, and 10–22 mice for each control group.

Antitumor activity was determined by comparing the median survival time of treated groups (T) with that of control groups (C), and expressed as T/C value. A T/C value of at least 125% is required to demonstrate activity.

Statistical analysis. The criterion of activity was survival [7].

Multiple comparisons were performed in order to compare:

– Each treated group with the corresponding control group;

– Each group treated with the combination of VCR and S 9788 with groups receiving VCR alone.

The log-rank test was used with threshold defined according to the Bonferroni method ($\alpha' = \alpha$/number of comparisons) [8].

Results

In vivo effects of S 9788 on reversal of drug resistance

Table 1 shows that VCR (0.5 mg/kg) administered alone on days 1, 5, 9 significantly increased the lifespan of P388-bearing mice (T/C value 190%). The same dose of VCR showed a much lower activity (T/C value 144%) against P388/VCR-bearing mice, as expected. S 9788 had no therapeutic effects alone, but significantly enhanced the antitumor activity of VCR (0.5 mg/kg) on P388/VCR. The effects were clearly dependent on the doses of S 9788. The T/C value obtained at the highest dose of S 9788 (300 mg/kg) was comparable with that observed in P388-bearing mice treated with VCR alone, indicating complete circumvention of resistance by combination VCR-S 9788 therapy. No excessive toxicity (defined as weight loss in excess of 4 g or death) was observed when S 9788 (50 mg/kg, 100 mg/kg, 200 mg/kg, 300 mg/kg) and VCR

Table 1. Effect of S 9788 on antitumor activity of vincristine in P388 and P388/VCR-bearing mice

P388					P388/VCR				
VCR (mg kg^{-1} day^{-1})	S 9788 (mg kg^{-1} day^{-1})	ΔP[a] D_5-D_1 (g)	Median survival day (range)	T/C[b] (%)	VCR (mg kg^{-1} day^{-1})	S 9788 (mg kg^{-1} day^{-1})	ΔP[a] D_5-D_1 (g)	Median survival day (range)	T/C[b] (%)
0	0	+1.3	10.1 (9–11)	100	0	0	+2.6	8.5 (8–10)	100
					0	50[c]	+1.7	9.0 (8–11)	106
0	100	+1.1	9.3 (9–10)	92	0	100[c]	+1.3	8.1 (7– 9)	94
0	200	+0.2	9.0 (9– 9)	90	0	200[c]	+1.0	8.1 (7– 9)	95
					0	300[c]	+0.4	8.2 (8–11)	96
0.5	0	–0.6	19.1[d] (17–20)	190	0.5	0	–1.1	12.3[d] (9–14)	144
					0.5	50	–1.1	14.3[d, e] (14–16)	167
0.5	100	–1.5	19.1[d] (18–20)	190	0.5	100	–1.5	15.7[d, e] (14–17)	183
0.5	200	–2.1	20.4[d] (18–22)	202	0.5	200	–1.7	16.8[d, e] (13–19)	196
					0.5	300	–1.6	17.0[d] (9–22)	199

[a] Mean body weight change in grammes between day 5 and day 1

[b] T/C = median survival time of treated animals/median survival time of controls × 100

[c] Treated only on days 1 and 5. Each other group of 10 CD_2F_1 mice transplanted IP on day 0 with 10^6 P388 or P388/VCR leukemic cells was treated IP with VCR and S 9788 on days 1, 5, 9. No deaths were observed in any group up to or on day 5 (toxicity day)

[d] Significant difference from the controls

[e] Significant difference from VCR alone

(Significance thresholds are $\alpha' = 0.007$ for treated groups with P388 leukemia and $\alpha' = 0.004$ for treated groups with P388/VCR leukemia)

Table 2. Effect of S 9788 on antitumor activity of vincristine in P388 and P388/VCR-bearing mice

P388						P388/VCR					
VCR (mg kg^{-1} day^{-1})	S 9788 (mg kg^{-1} day^{-1})	ΔP^a D_5-D_1 (g)	Median survival day (range)		T/C[b] (%)	VCR (mg kg^{-1} day^{-1})	S 9788 (mg kg^{-1} day^{-1})	ΔP^a D_5-D_1 (g)	Median survival day (range)		T/C[b] (%)
0	0	+2.3	9.6	(9–10)	100	0	0	+2.0	8.7	(8–10)	100
							50	+1.4	9.1	(8–10)	104
							100	+0.4	8.4	(8–9)	97
							200	–0.8	8.3	(8–9)	95
0.1	0	+0.7	15.9[c]	(14–18)	165	0.1	0	+0.8	10.0[c]	(9–12)	115
0.2	0	–0.4	15.7[c]	(15–18)	163	0.2	0	+0.3	10.4[c]	(9–12)	119
						0.1	50	–0.4	13.6[c, d]	(12–15)	156
						0.1	100	–0.9	14.2[c, d]	(13–15)	163
						0.1	200	–1.0	15.3[c, d]	(10–16)	175
						0.2	50	–1.4	15.6[c, d]	(13–16)	179
0.2	100	–1.7	18.7[c, d]	(12–20)	195	0.2	100	–2.1	15.8[c, d]	(13–18)	181
						0.2[e]	200	–1.8	9.6	(8–20)	110

[a] Mean body weight change in grammes between day 5 and day 1

[b] T/C = median survival time of treated animals/median survival time of controls $\times 100$

[c] Significant difference from the controls

[d] Significant difference from VCR alone

(Significance thresholds are $\alpha' = 0.025$ for treated groups with P388 leukemia and $\alpha' = 0.003$ for treated groups with P388/VCR leukemia)

[e] Treated daily for only 8 days. Each other group of 10 CD_2F_1 mice transplanted IP on day 0 with 10^6 P388 or P388/VCR leukemic cells was treated IP with VCR and S 9788 for 9 days. No deaths were observed in any group up to or on day 5 (toxicity day)

(0.5 mg/kg) were administered alone or in combination. Previous experiments in which the same criteria were used to judge treatment toxicity showed that the optimal dose of VCR alone (given on days 1, 5, 9) for the treatment of P388-bearing mice was 1–2 mg kg^{-1} day^{-1}. Associations of S 9788 with these higher doses of VCR were found to be toxic in both P388 and P388/VCR-bearing mice (data not shown).

In order to examine the effects of dosage of S 9788 and VCR on complete reversal of resistance, another treatment schedule was tested. As indicated in Table 2, VCR (0.1 mg/kg–0.2 mg/kg) administered IP daily for 9 days showed no activity in P388/VCR-bearing mice (T/C values 115–119%). A markedly improved therapeutic effect was observed when VCR was coadministered with S 9788. T/C values of 156–181% were obtained when S 9788 (50–200 mg/kg) was associated with VCR (0.1 mg/kg–0.2 mg/kg). Toxic effects appeared for the association of S 9788 at 200 mg/kg and VCR at 0.2 mg/kg, with a T/C value of 110%. The increases in life span in P388/VCR-bearing animals treated with combination therapy (56–81%) were equivalent to those observed for P388-bearing mice treated with VCR alone, indicating a potent ability of S 9788 to reverse resistance in this second schedule of administration. In addition, S 9788 was also effective in enhancing therapeutic effects of VCR in the treatment of P388-bearing mice. When S 9788 (100 mg/kg) was associated with VCR at 0.2 mg/kg (T/C value 195%) an increase in life span over that obtained with treatment of P388-bearing animals with VCR alone (T/C value 163%) was obtained.

Discussion

In the present study we showed that S 9788, a new triazinoaminopiperidine derivative, was a potent reversant of vincristine resistance in the murine leukemia P388/VCR model. When administered IP with VCR on days 1, 5 and 9, S 9788 caused an increase in the life span of P388/VCR-bearing mice, which was dose dependent. Complete circumvention of vincristine resistance was obtained with the highest doses of S 9788 (200 and 300 mg/kg), as observed by comparing the T/C values obtained after treatment of sensitive P388-bearing mice with VCR alone with those obtained after treatment of P388/VCR-bearing mice with VCR-S 9788 combination therapy. These results are remarkable because, while many pharmacological agents have been found to completely overcome drug resistance in vitro, the number of reports showing such a phenomenon in vivo are rather limited [3, 5]. The principal reason for the lack of activity of numerous chemosensitizers in vivo results from their high toxicity and the difficulty of maintaining active doses without causing important side effects. Such effects may be due to the interaction of MDR modulators with their target, P-glycoprotein, which has been demonstrated to be highly expressed in several normal tissues [2, 14]. In this respect, problems of non-selective antitumor toxicity of combinations in which chemosensitizers are associated with anticancer drugs must be accurately considered. In the case of S 9788, the data presented here support the notion of selective cytotoxicity of S 9788 – vincristine in the P388/VCR model. However, we observed a similar effect to that reported with other

modulators using the same model system [12, 13] but different treatment protocols, in that toxicity was seen with combinations involving higher doses of VCR which, at least in our experiments, were optimal for treatment of sensitive tumours.

To optimize this phenomenon a second schedule of administration of VCR-S9788 (injection daily on days 1–9) was tested. A complete reversion of resistance was obtained for cumulated doses of VCR (0.1 mg kg^{-1} day^{-1} $\times 9$) lower than those used in the above treatment (0.5 mg kg^{-1} $day^{-1} \times 3$) associated with increased doses of S 9788 (100 mg/kg $\times 9$ versus 200 mg/kg $\times 3$). These results show, as reported previously, that the combination of relatively low doses of antitumor agents with as high a dose of modulator as possible is effective in reversing drug resistance and in having selectivity towards tumor cells [13]. Significant effects were also observed in sensitive P388-bearing mice with a combination of VCR and S 9788 administered daily from day 1 to day 9. Other modulators, such as verapamil, have previously been shown to potentiate the activity of anticancer drugs on sensitive experimental tumors [4, 13, 16].

These results could be explained by an increased accumulation of anticancer drugs in sensitive cells. Another explanation, which does not rule out the former, is that antitumor agent-modulator combination therapy would be effective in preventing the emergence of a population of resistant tumors cells in the sensitive tumor [16].

S 9788 is a novel compound, which belongs to a novel class of agents able to reverse MDR. Its potency to completely reverse multidrug resistance in vivo has been clearly demonstrated in this study. Its mechanism of action is currently under investigation.

Further studies will be required for further exploration of the potential of S 9788 for clinical use, including the influence of drug formulation and time between the administration of the two drugs. Experiments on the more predictive tumor xenograft models are being initiated, and pharmacokinetic studies are currently being considered.

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