POTENTIATION OF CHEMOTHERAPEUTIC EFFECT OF VINCRIStINE IN VINCRIStINE RESISTANT TUMOR BEARING MICE BY CALMODULIN INHIBITOR CLOMIPRAMINE

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Clomipramine, which is used as antidepressant and possesses calmodulin inhibitory activity, circumvented partly the vincristine resistance *in vivo*. Although vincristine alone at 30–200 $\mu\text{g}/\text{kg}$ did not confer a significant therapeutic effect in vincristine resistant P388 leukemia (P388/VCR)-bearing mice, clomipramine at doses of 20 to 50 mg/kg administered daily for 10 d with vincristine enhanced the chemotherapeutic effect of vincristine in P388/VCR-bearing mice. Approximately a 30% increase in life span occurred. Although the circumvention of vincristine resistance was not achieved perfectly, it could be speculated that more than 98–99% of vincristine resistant tumor cells which could not be killed by vincristine alone could be killed by this approach.

Keywords — drug resistance; clomipramine; calmodulin inhibitor; vincristine; P388 leukemia

One of the major reasons for treatment failure in cancer chemotherapy is the appearance and proliferation of drug-resistant tumor cells during the course of treatment.^{1–3)} The development of new modality to circumvent the drug resistance in tumor cells could be very beneficial in the present cancer chemotherapy.

Recent findings from this laboratory describe an enhancement of vincristine (VCR) cytotoxicity in P388 leukemia and its VCR-resistant subline by the calcium influx blocker verapamil.⁴⁾ Subsequent paper describes that the similar effect has been obtained by other calcium influx blocker and calmodulin inhibitors.⁵⁾ The circumvention of vincristine and Adriamycin resistance *in vivo* has been unsuccessful, however, the approach using calcium influx blockers could partly circumvent the vincristine and Adriamycin resistance *in vivo*.^{4,6)} Calmodulin inhibitors also circumvented the vincristine and Adriamycin resistance *in vitro*,⁵⁾ however, the effect of these agents *in vivo* are not impressive, presum-

ably because of their toxicity toward host animals. Among the examined calmodulin inhibitors, we found that clomipramine enhanced the chemotherapeutic effect of vincristine *in vivo*. Clomipramine is an antidepressant,^{7,8)} and has been reported to possess a calmodulin inhibitory activity.⁵⁾ Clomipramine can circumvent vincristine and Adriamycin resistance *in vitro*.⁵⁾ In this report we describe the effect of clomipramine on the circumvention of vincristine resistance *in vivo*.

One million of vincristine resistant P388 leukemic cells (P388/VCR, obtained from National Cancer Institute, NIH, Bethesda, Md. U.S.A.) were transplanted *i.p.* into CDF₁ mice (Charles River Japan) as described previously.⁴⁾ Clomipramine (provided by Ciba-Geigy Japan Ltd.) and vincristine were dissolved in 0.9% NaCl solution, and both drugs were mixed, and the mixture was administered at a constant rate of 0.02 ml/g body weight *i.p.* daily for 10 d starting from the day after the tumor inoculation.

Antitumor activity was expressed by (a) T/C (%), the mean survival time of the treated group was divided by that of control group of mice; (b) T/V, at each dosage of vincristine, the mean survival time of the treated group was divided by that of the group treated with vincristine alone.⁴⁾ Five mice were used in each experimental group.⁴⁾

Vincristine administered daily for 10 d start-

ing from day 1 increased the life span of P388 leukemia bearing mice. T/C values were 150, 173, and 223% at vincristine dosages of 30, 100, and 200 $\mu\text{g/kg}$, respectively (Table I, Exp. 1)

Vincristine given according to the schedule above showed no therapeutic effect in P388/VCR-bearing mice, except at a dosage of 200 $\mu\text{g/kg}$ where a slightly higher T/C value (113%) was obtained. Clomipramine alone also

TABLE I. *Effect of Clomipramine on Antitumor Activity of VCR in P388-, and P388/ VCR- bearing Mice*

	Drug and dosage	Survival time (d)	T/C (%)	T/V ^{a)} (%)
Exp. 1	P388 bearing mice			
	Control	10.0 $\pm$ 0.9 ^{b)}	100	
	VCR (30 $\mu\text{g/kg}$)	15.0 $\pm$ 1.5	150 ^{c)}	
	VCR (100 $\mu\text{g/kg}$)	17.3 $\pm$ 1.7	173 ^{c)}	
	VCR (200 $\mu\text{g/kg}$)	22.3 $\pm$ 3.7	223 ^{c)}	
Exp. 2	P388/VCR bearing mice			
	Control	11.0 $\pm$ 0	100	
	Clomipramine (50mg/kg)	10.6 $\pm$ 0.5	96	
	Clomipramine (40mg/kg)	11.6 $\pm$ 1.1	105	
	VCR (200 $\mu\text{g/kg}$)	12.4 $\pm$ 1.0	113 ^{c)}	100
	+Clomipramine (50mg/kg)	12.8 $\pm$ 1.3	116 ^{c)}	103
	+Clomipramine (40mg/kg)	15.2 $\pm$ 0.5	138 ^{c)}	123 ^{d)}
	+Clomipramine (30mg/kg)	14.2 $\pm$ 1.1	129 ^{c)}	115 ^{d)}
	+Clomipramine (20mg/kg)	13.6 $\pm$ 0.9	124 ^{c)}	110
	VCR (100 $\mu\text{g/kg}$)	10.8 $\pm$ 1.3	98	100
	+Clomipramine (50mg/kg)	12.0 $\pm$ 3.0	109	111
	+Clomipramine (40mg/kg)	13.6 $\pm$ 1.1	124 ^{c)}	126 ^{d)}
	+Clomipramine (30mg/kg)	14.4 $\pm$ 1.8	131 ^{c)}	133 ^{d)}
	+Clomipramine (20mg/kg)	14.4 $\pm$ 2.0	131 ^{c)}	133 ^{d)}
	VCR (30 $\mu\text{g/kg}$)	10.6 $\pm$ 2.1	96	100
	+Clomipramine (50mg/kg)	10.2 $\pm$ 4.6	93	96
	+Clomipramine (40mg/kg)	13.8 $\pm$ 1.5	126 ^{c)}	130 ^{d)}
	+Clomipramine (30mg/kg)	12.0 $\pm$ 0.7	109 ^{c)}	113
	+Clomipramine (20mg/kg)	11.8 $\pm$ 0.8	107	111

Each group of 5 CDF₁ mice was given i.p. implants of 10⁶ cells of P388 or P388/ VCR leukemia on day 0, and drugs were given i.p. daily from day 1 to 10.

a) T/V, at each VCR dosage, the mean survival time of the treated group was divided by the mean survival time of the group of mice treated with VCR alone.

b) Mean $\pm$ S.D.

c) Statistically significant ($p < 0.05$) by Student's *t* test as compared with that of the control experiments.

d) Statistically significant ($p < 0.05$) by Student's *t* test as compared with that of mice treated with VCR alone at each dosage of VCR.

did not confer a significant therapeutic effect against P388/VCR-bearing mice.

However, clomipramine (20–50 mg/kg) given 10 times with vincristine (30–200 $\mu\text{g/kg}$) significantly increased the life span of the P388/VCR-bearing mice. Especially notable was a 30% increase in life span which was observed when clomipramine (20–40 mg/kg) was administered with vincristine (100 $\mu\text{g/kg}$). The combination of two drugs at other doses also significantly increased the life span of the mice, although clomipramine at 50 mg/kg in combination with vincristine rather reduced the chemotherapeutic effect. The enhanced T/C values, however, were less than those obtained in P388-bearing mice treated with vincristine alone at 30 $\mu\text{g/kg}$ (T/C=150%). These results indicate that the circumvention of VCR resistance *in vivo* was not perfect, however, VCR resistance could be partly circumvented in the P388/VCR bearers when a larger amount of vincristine was given with clomipramine. At the most effective combinations, approximately 3–4 d increase in life span was obtained in this regimen. This type of leukemia (P388 or P388/VCR) multiplies approximately twice a day *in vivo*. Actually, according to Schabel *et al.*,⁹⁾ one day increase in life span in mice bearing P388 leukemia corresponds approximately to the reduction of tumor burden to 1/5. Although the present treatment regimen was not the same with that of Schabel *et al.*, three to four days increase in life span observed here might correspond to the therapeutic effect of more than 98–99% kill of vincristine resistant tumor cells attainable by vincristine in combination with clomipramine.

The mechanism of the enhancement of vincristine cytotoxicity by calcium influx blockers and calmodulin inhibitors is the inhibition of vincristine efflux function of tumor cells, especially of vincristine resistant tumor cells.^{4,5)} Vincristine and Adriamycin accumulated greatly in tumor cells, especially in resistant tumor cells, re-

sulting in the marked enhancement of drug cytotoxicity in drug resistant tumor cells.^{4,5)} The approach described here has some possibility to circumvent the drug resistance clinically. At present we are speculating upon the possibility of identifying clinically effective calmodulin inhibitors to achieve better destruction of resistant tumor cells.

REFERENCES

- 1) F.M.Schabel, Jr.: Concepts for systemic treatment of micrometastases, *Cancer*, **35**, 15–24 (1975).
- 2) F.M.Schabel, Jr., H.E.Skipper, M.W.Trader, W.R.Laster, Jr., T.H.Corbett, and D.P.Griswold, Jr.: Concepts for controlling drug-resistant tumor cells. In: H.T.Mouridsen, and T.Palshof (eds.), *Breast Cancer Experimental and Clinical Aspects*, Oxford, Pergamon Press, 1980, pp. 199–211.
- 3) H.E.Skipper, F.M.Schabel, Jr., and H.H.Lloyd: Experimental therapeutics and kinetics: Selection and overgrowth of specifically and permanently drug-resistant tumor cells, *Semin. Hematol.*, **15**, 207–219 (1978).
- 4) T.Tsuruo, H.Iida, S.Tsakagoshi, and Y.Sakurai: Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil, *Cancer Res.*, **41**, 1967–1972 (1981).
- 5) T.Tsuruo, H.Iida, S.Tsakagoshi, and Y.Sakurai: Increased accumulation of vincristine and adriamycin in drug-resistant tumor cells following incubation with calcium antagonists and calmodulin inhibitors, *Cancer Res.*, **42**, 4730–4733 (1982).
- 6) T.Tsuruo, H.Iida, M.Nojiri, S.Tsakagoshi, and Y.Sakurai: Circumvention of vincristine and Adriamycin resistance *in vitro* and *in vivo* by calcium influx blockers, *Cancer Res.*, submitted (1982).
- 7) R.M.Levin, and B.Weiss: Selective binding of antipsychotics and other psychoactive agents to the calcium-dependent activator of cyclic nucleotide phosphodiesterase, *J. Pharmacol. Exp. Ther.*, **208**, 454–459 (1979).
- 8) B.Weiss, and R.M.Levin: Mechanism for selectively inhibiting the activation of cyclic nucleotide phosphodiesterase and adenylylase by antipsychotic agents, *Adv. in Cyclic Nucleotide Res.*, **9**, 285–303 (1978).
- 9) F.M.Schabel, Jr., D.P.Griswold, Jr., W.R.Laster, Jr., T.H.Corbett, and H.H.Lloyd: Quantitative evaluation of anticancer agent activity in experimental animals, *Pharmac. Ther.*, **1**, 411–435 (1977).