SHORT COMMUNICATION
Clinically relevant concentrations of verapamil do not enhance the sensitivity of human bone marrow CFU-GM to adriamycin and VP16

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Bone marrow toxicity is a major dose-limiting factor in the clinical use of adriamycin (doxorubicin) and VP16 (VP16-213, etoposide). While the calcium antagonist verapamil has been reported to enhance the sensitivity of a number of animal (Tsuruo et al., 1982; Ramu et al., 1984; Yalowich & Ross, 1984; Tsuruo et al., 1985; Supino et al., 1986) and human (Tsuruo et al., 1983a; Rogan et al., 1984; Merry et al., 1986a, Merry et al., 1987; Slater et al., 1986; Twentyman et al., 1986) tumours to these cytotoxic agents in vitro, the effects of verapamil on the cytotoxic drug sensitivity of bone marrow has not been extensively studied.

Using a clonogenic assay, we have studied the effect of verapamil at a range of concentrations (including concentrations achievable in vivo) on the sensitivity of human bone marrow granulocyte-macrophage stem cells (CFU-GM) to adriamycin and VP16. Our findings are presented in this short report.

Marrow was aspirated from patients with iron or B12/folate deficiency, whose haemopoiesis could be normalised in vitro. Marrow was spun over Ficoll-diatrizoate (SG1.077) at 400 g for 30 min and light density marrow cells (LDMCs) were harvested from the interface. Cells were washed once in RPMI-1640 (Gibco UK).

Lymphocyte conditioned medium was produced by producing light density peripheral blood mononuclear cells (PBNCs) from normal subjects obtained using Ficoll-diatrizoate separation (as above). PBMCs were adjusted to a concentration of 1 x 10³/ml in RPMI-1640 medium and supplemented with 10 mM HEPES and L-glutamine (Gibco UK) and 0.5% L-glutamine. Cell suspensions were incubated at 37°C for 6 days in tissue culture flasks (Nunclon, Denmark) in a 5% CO₂ atmosphere and 98% humidity with phytohaemagglutinin-P (Difco, UK) 0.75% v/v.

After incubation, supernatants were sterile filtered (Milllex 0.22 μm filters, Millipore UK Ltd.), pooled, diluted 1:4 in RPMI-1640 medium and stored at -20°C.

VP16 (Mol. wt 588.6) was obtained as a pure powder from Dr Dale Stringfellow, Bristol Myers Co. Ltd., Syracuse, USA. The drug was initially dissolved in 50% ethanol, then diluted in normal saline. A solvent control with ethanol at final concentration of 0.025% was included. Adriamycin (Mol. wt 380) was obtained from Farmitalia, Carlo Erba Ltd., Barnet, Herts and dissolved in saline. Verapamil (Mol. wt 491.1) was obtained from Abbott Laboratories Ltd., Queensborough, Kent and diluted in saline.

LDMCs to be cultured were incubated with or without the addition of drugs (Table I) for 1 h at 37°C in 2 ml RPMI-1640 medium. Post incubation cells were washed twice then set up in semisolid culture at a concentration of 1 x 10³/ml. The addition of lymphocyte conditioned medium was obligatory for CFU-GM colony formation.

LDMCs were cultured in supplemented Dulbecco’s modified Eagles MEM (Gibco UK) with 0.8% methyl cellulose (4,000 cp Fluka, UK), heat inactivated foetal calf serum and L-glutamine (60%, 20% and 0.5% of final culture volume respectively). The remaining volume comprised pooled, diluted lymphocyte conditioned medium. Cultures were performed in Multilwell dishes (Costar, Cambridge, Mass., USA) in quadruplicate. CFU-GM colonies were scored after 7 days incubation at 37°C in a humidified atmosphere of 5% CO₂. A colony was defined as containing 40 or more cells.

The results of our cloning experiments are shown in Table I. A total of 10 bone marrow specimens were assayed.

In preliminary studies (subjects 5 and 6) colony number was determined in the presence and absence of the solvent used to dissolve the VP16 (i.e. 0.025% ethanol, final concentration). In each case no effect of solvent was noted and in subsequent experiments only solvent-containing controls were set up. The effect of verapamil alone on colony number was also determined (8 cases at 6.6 μM, 4 cases at 3.3 μM, 4 cases at 1.5 μM and 8 cases at 0.66 μM). In no instance was colony number reduced by more than 10% compared to solvent controls.

The bone marrow specimens exhibited a range of sensitivities in vitro to the concentrations of cytotoxic drugs used in these studies. In the case of adriamycin, colony number was reduced by between 24% and 78% (10 cases). For VP16 colony number was reduced between 19% and 76% (10 cases). The mean reduction in colony number was 56% for adriamycin and 40% for VP16. Furthermore the bone marrow specimens which showed the greatest sensitivity to adriamycin (subjects 1, 3, 4, 6, 8 and 10) also showed the greatest sensitivity to VP16.

Verapamil (at non-cytotoxic concentrations of 3.3–6.6 μM) did enhance the sensitivity of human bone marrow CFU-GM to adriamycin and VP16, but at the clinically relevant doses of 0.66–1.5 μM little effect was noted. Specifically, in comparison to adriamycin treatment alone, colony number was reduced more than 20% to 6.6 μM verapamil plus adriamycin in 3/8 cases (subjects 4, 5 and 6); by 3.3 μM verapamil plus adriamycin in 2/4 cases (subjects 3 and 4); by 1.5 μM verapamil plus adriamycin on 0/4 cases and by 0.66 μM verapamil plus adriamycin in 0/8 cases. For VP16, the corresponding results are 7/8 cases (subjects 1, 2, 3, 4, 5, 6, 7, 8 and 10) at 6.6 μM verapamil; 3/4 cases (subjects 1, 3 and 4) at 3.3 μM verapamil; 0/4 cases at 1.5 μM verapamil and 1/8 cases (subject 10) at 0.66 μM verapamil. Furthermore in some cases (subjects 1, 2 and 10) concentrations of verapamil that enhanced sensitivity to VP16 did not do so for adriamycin. Further experiments (using a more extensive series of specimens and with a range of cytotoxic drug concentrations) would be required to confirm the generality of this latter observation.

There have been a limited number of previous studies of the effect of verapamil on the sensitivity of human bone marrow to cytotoxic drugs. Robinson et al. (1985) showed that verapamil at a concentration of 23 μM had no effect on the sensitivity of human bone marrow to melphalan. Fine et al. (1987) showed that 2.2 μM verapamil did not enhance

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Received 3 January 1988; and in revised form 8 March 1988.
sensitivity to adriamycin or vinblastine and Yalowich et al. (1985) showed that 2.5-40 \mu M verapamil enhanced sensitivity to VP16, but not to adriamycin and vincristine. Our data are broadly in agreement with those of Fine et al. and with those of Yalowich et al. for VP16, but conflict with Yalowich’s results for adriamycin. Since conditions of drug treatment (1 h, 37°C) in this latter study were similar to our own the different results are most likely due to differences in the conditions used for cloning. Most notably, the use of different colony stimulating factors may be important in the selection of different populations to cells to grow to form colonies.

While plasma levels of verapamil of up to 10 \mu M may be achieved clinically by intravenous infusion (Ozols et al., 1984) these are associated with significant cardiovascular toxicity. Recent clinical trials (Benson et al., 1985; Cantwell et al., 1985) have however shown that steady state concentrations of verapamil in plasma of 0.5–1 \mu M can be maintained with limited toxicity. In the context of enhancement of drug sensitivity this concentration of verapamil is at the lower end of the dose-response curve, but some studies (Yalowich & Ross, 1984; Slater et al., 1986; Supino et al., 1986) do indicate that verapamil may enhance sensitivity to adriamycin and VP16 at concentrations of 1–2 \mu M in vivo. These concentrations of verapamil in vivo have also been reported to enhance tumour cell sensitivity to vinca alkaloids (Tsuruo et al., 1981; Tsuruo et al., 1983a; Simmonds et al., 1986) and daunorubicin (a structural analogue of adriamycin: Slater et al., 1982).

In vivo, using animal ascites tumour models, verapamil has been reported to enhance sensitivity to VP16 (Slater et al., 1986), daunorubicin (Slater et al., 1982) and vincristine (Tsuruo et al., 1981). In these studies drug treatment was administered intraperitoneally. It is not yet known whether enhancement of drug sensitivity in vivo by verapamil is a general phenomenon for solid tumours, but preliminary data indicate that it may be. Tsuruo et al. (1983c) have shown that administration of vincristine plus verapamil increases the survival of mice bearing colon adenocarcinoma growing intraperitoneally compared to treatment with vincristine alone. Furthermore verapamil has been reported to enhance the sensitivity of a subcutaneously-growing murine fibrosarcoma to melphanal (Robinson et al., 1985), of a human neuroblastoma xenograft to cisplatinum (Ikeda et al., 1987) and of human lung cancer xenograft to vincristine (Mattern et al., 1987). Our preliminary data using human lung cancer xenografts (Merry et al., 1986b) and the subcutaneously growing murine Ridgeway osteogenic sarcoma (ROS, unpublished) have also shown that verapamil is able to increase sensitivity to VP16 and, in the case of the ROS tumour, vincristine and actinomycin D. In this context it is important to note that maximum plasma concentrations of 1.6 \mu M were obtained in our xenograft study.

In summary, our data indicates that verapamil at concentrations of 0.66–1.5 \mu M does not enhance the sensitivity of human bone marrow CFU-GM to adriamycin and VP16. Other reports have shown that (a) 1–2 \mu M verapamil enhances tumour sensitivity in vivo, (b) verapamil (at peak plasma concentrations of 1.6 \mu M) enhances tumour sensitivity in vitro and (c) plasma concentrations of 0.5–1 \mu M can be maintained in humans with minimal toxicity. Verapamil may therefore enhance cytotoxic drug sensitivity in tumour tissue at clinically achievable concentrations without increasing marrow toxicity. Clinical trials to determine the efficacy of verapamil in overcoming tumour drug resistance would appear to be justified. Our observations that human bone marrow CFU-GM have a range of sensitivities to adriamycin and VP16 and that, in some cases, sensitivity to VP16 is enhanced at concentrations of verapamil which do not enhance adriamycin sensitivity may also have important clinical consequences; although further studies are required.

The authors would like to thank the Cancer Research Campaign for financial support. Our thanks are also due to Mrs M. McLeod and Mrs M. Jenkins for secretarial assistance.

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### Table 1

| Subject | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---------|---|---|---|---|---|---|---|---|---|---|
| Solvent control | 144±16 | 297±6 | 296±6 | 240±15 | 207±10 | 218±17 | 289±15 | 253±9 | 319±12 | 309±13 |
| 0.6 \mu M VP16 | 139±3 | 278±16 | 270±17 | 233±23 | 198±9 | 212±8 | — | — | — | — |
| 3.3 \mu M VP16 | 130±15 | 293±1 | 267±17 | 223±5 | — | — | — | — | — | — |
| 1.5 \mu M VP16 | 146±14 | 279±2 | 295±7 | 236±17 | — | — | — | — | — | — |
| 0.66 \mu M VP16 | 150±12 | 288±3 | 290±16 | 226±15 | — | — | — | — | — | — |
| 0.6 \mu M ADR* | 33±5 | 177±35 | 91±15 | 57±8 | 154±10 | 75±9 | 144±8 | 121±9 | 199±3 | 130±10 |
| 0.6 \mu M ADR+6.6 \mu M VP16 | 33±8 | 186±34 | 94±11 | 30±8 | 107±7 | 22±9 | — | — | — | — |
| 0.6 \mu M ADR+3.3 \mu M VP16 | 40±4 | 171±34 | 68±5 | 33±3 | — | — | — | — | — | — |
| 0.6 \mu M ADR+1.5 \mu M VP16 | 32±2 | 178±39 | 93±13 | 65±17 | — | — | — | — | — | — |
| 0.6 \mu M ADR+0.66 \mu M VP16 | 37±6 | 210±7 | 88±18 | 59±4 | — | — | — | — | — | — |
| 50 \mu M VP16 | 35±18 | 211±14 | 129±12 | 144±14 | 168±4 | 136±11 | 194±8 | 158±10 | 231±8 | 172±17 |
| 50 \mu M VP16+6.6 \mu M VP16 | 14±26 | 166±6 | 71±11 | 108±55 | 115±12 | 89±14 | — | — | — | — |
| 50 \mu M VP16+3.3 \mu M VP16 | 24±3 | 193±26 | 77±8 | 114±12 | — | — | — | — | — | — |
| 50 \mu M VP16+1.5 \mu M VP16 | 28±5 | 227±18 | 131±10 | 115±11 | — | — | — | — | — | — |
| 50 \mu M VP16+0.66 \mu M VP16 | 37±5 | 215±16 | 130±14 | 153±6 | — | — | — | — | 200±9 | 164±20 | 237±6 | 100±4 |

*a*Results expressed as MEAN±s.d.; *\(^{\ast}\)*Indicates colony number not determined; *Verapamil; *Adriamycin.
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