Cancer Chemother Pharmacol (1982) 7: 123–126

Cancer Chemotherapy and Pharmacology

Interaction of VP16-213 with the DNA Repair Antagonist Chloroquine

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Introduction

VP16-213 is a semi-synthetic derivative of podophyllotoxin with significant antitumour activity both in animals and in man [1, 11, 17, 19]. Although structurally closely related, VP16-213 and podophyllotoxin have been shown to have different mechanisms of action [13–14]. Podophyllotoxin acts as a classical spindle poison and arrests cells in mitosis [5]. By contrast VP16-213 acts at an earlier stage in the cell cycle, and does not interfere with microtubule assembly [9]. Using alkaline sucrose gradient sedimentation profiles Loike and Horwitz [14] showed that VP16-213 produced single stranded breaks in HeLa cell D.N.A. The breaks were repairable and on removal of VP16-213 from the system the cells recovered.

The 4 amino quinalone antimalarial drug chloroquine has been shown to inhibit the repair of single strand DNA breaks caused by methyl methanesulfonate in cultured rat liver cells [15]. It was of interest to study in vivo, the interaction of VP16-213 with chloroquine using a murine lymphoma model. As podophyllotoxin is not active against TLX 5 lymphoma, vincristine, a drug with a similar mode of action, [10] was chosen as the control as it is not known to act through damage to DNA.

Materials and Methods

Animals and Cell Lines. Female CBA/Ca mice weighing 18–22 grammes were obtained from Olac Ltd., Bicester, Oxfordshire, GB. They were kept in cages of five and allowed food and water ad libitum. The TLX5 murine ascitic tumour was obtained from the Institute of Cancer Research, London. It is a T cell lymphoma produced by thymic irradiation of CBA/Ca mice. The cells are passaged in suspension by intraperitoneal injection every seven days. For experiments a suspension containing 1×10^5 cells in 0.1 ml normal saline was injected subcutaneously in the region of the right thigh muscles of the mice. If left untreated control animals die of disseminated lymphoma about 10 days after inoculation.

Chemicals. VP16-213 was provided by Bristol-Myers, Slough, Buckinghamshire, GB; Vincristine was purchased from Eli Lilly and Company Ltd., Basingstoke, Hampshire, GB. Chloroquine sulphate was purchased from May and Baker LTD, Dagenham, Essex, GB. All drugs were diluted in normal saline and administered in 0.1 ml aliquots.

Experimental Design. All experiments were carried out using at least 10 animals per dose level. On day 1, animals were inoculated subcutaneously with TLX5 lymphoma cells as described above.

On day 3 the test drugs were administered intraperitoneally. Where two drugs were administered they were given simultaneously via separate syringes. In two experiments chloroquine was given either 6 h before or 6 h after VP16-213. Experiments were performed in duplicate and results were expressed as mean percent increased life span (ILS) where:

$$\text{ILS} = \frac{\text{Survival (in days) of treated animals}}{\text{Survival in days of untreated controls}} \times 100 .$$

Preliminary toxicity studies were carried out in non tumour bearing animals to assess the toxicity of drug combinations and to choose working dosages for subsequent experiments. Toxicity was manifest as:

a) Acute toxicity – early death of mice within 1 h of inoculation.

b) Chronic toxicity – the mean percentage weight change over the 5 days following drug administration.

Statistical significance was assessed at the 0.05 level according to Student's test for analysis.

Results

Toxicity Studies

Acute and chronic toxicity studies in non tumour bearing animals are shown in Table 1. When used alone VP16-213 at doses of 25 and 50 mg/kg did not produce detectable toxicity while at a dose of

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0344-5704/82/0007/0123/$01.00

Table 1. Acute and chronic toxicity of VP16-213 and chloroquine in non tumour bearing mice

		Acute toxicity	Chronic toxicity % change weight over 5 days
VP16-213	25 mg/kg	–	+ 3.5%
	50 mg/kg	–	+ 4%
	100 mg/kg	–	– 22%**
Chloroquine	50 mg/kg	–	+ 3%
	100 mg/kg	100%	–
VP16-213 + Chloroquine	25 mg/kg + 50 mg/kg	–	– 2%
VP16-213 + Chloroquine	50 mg/kg + 50 mg/kg	40%	– 6.5%*

* Toxicity in remaining animals
** All died within 8 days (mean 6.7)

100 mg/kg it produced marked weight loss and the death of all animals within 8 days (mean 6.7). Chloroquine at a dose of 50 mg/kg did not produce toxicity but when the dose was doubled all animals died within a few minutes from seizures. The combination of VP16-213 25 mg/kg with chloroquine 50 mg/kg did not produce significant toxicity; however when the dose of VP16-213 was doubled to 50 mg/kg (which does not cause toxicity when used alone) both acute and chronic toxicity was manifest suggesting a degree of additive toxicity. In an attempt to avoid toxicity in subsequent experiments chloroquine was used at a dose of 40 mg/kg which was chosen as the maximum dose that could be administered without the risk of acute toxicity. Used at this dose no animals died of seizures and weight loss over the first 5 days was not significant.

VP16-213 and Chloroquine in Tumour Bearing Animals

Chloroquine alone did not have any significant anti-tumour activity against TLX 5 lymphoma (Table 2). VP16-213 alone at low dosage (6.25 and 12.5 mg/kg) had a slight but reproducible antitumour activity producing an ILS of 9% and 30% respectively ($P = < 0.05$) (Fig 1). When used in combination with chloroquine 40 mg/kg the improvement in ILS was marked (78% and 76% respectively) ($P = < 0.01$).

The effect of varying the timing of chloroquine administration is shown in Table 3. Again chloroquine and VP16-213 given simultaneously markedly improved ILS. When chloroquine was administered 6 h prior to VP16-213 ILS was increased but to a lesser degree. However, when the drug was administered 6 h following VP16-213 administration no improvement in ILS occurs.

Figure 2 shows the dose response curve of VP16-213 alone and in combination with chloroquine to maximally to tolerated doses.

The maximum ILS produced by VP16-213 alone is 96% (40 mg/kg). The maximum ILS produced by

Table 2. Antitumour activity and toxicity of chloroquine alone in tumour bearing mice

Dose of chloroquine (mg/kg)	% ILS	% change in weight
Controls	–	+ 3%
20	+ 1%	+ 2%
40	– 2%	+ 1%
60	*	–

* Died of acute toxicity

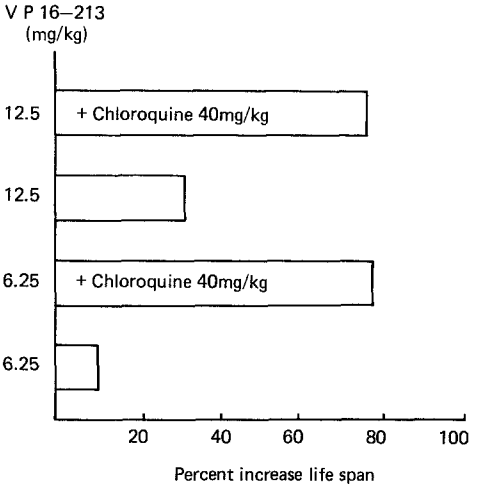

Fig. 1. Combination of VP16-213 in low dosage with chloroquine in tumour bearing animals

Table 3. Effect of giving chloroquine either 6 h before or 6 h after VP16-213

	Dose of VP16-213 (mg/kg)	
	6.25	12.5
	% ILS	% ILS
VP16-213 alone	8%	27%
VP16-213 chloroquine together	73%	83%
VP16-213 chloroquine 6 h before	38%	56%
VP16-213 chloroquine 6 h after	14%	30%

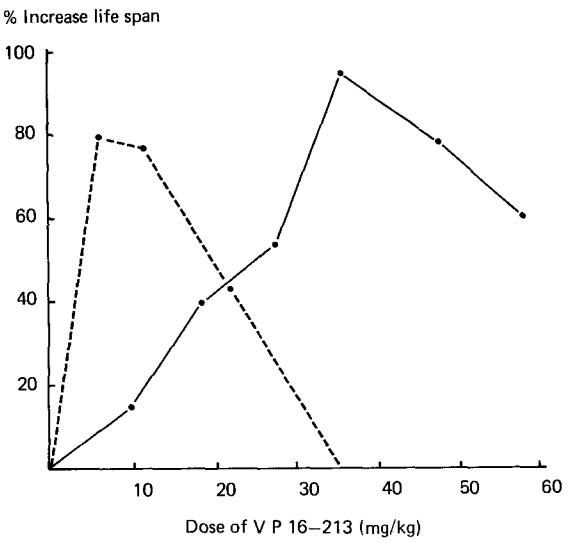

Fig. 2. Combination of VP16-213 and chloroquine in tumour bearing animals (●——●) VP16-213 + chloroquine; (●– – –●) VP16-213 alone

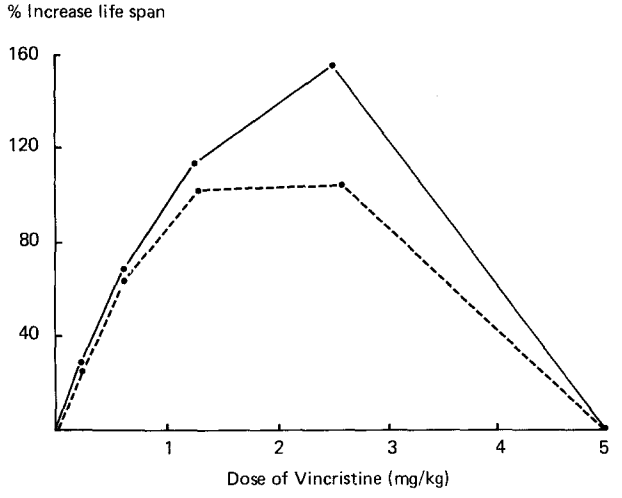

Fig. 3. Combination of Vincristine and chloroquine in tumour bearing animals. (●——●) Vincristine + chloroquine; (●– – –●) Vincristine alone

the combination of VP16-213 with chloroquine is 78% (VP16-213 6.25 mg/kg and Chloroquine 40 mg/kg). With higher doses of VP16-213 the ILS decreased presumably due to toxicity. It should be noted that while the combination of VP16-213 with chloroquine used in low doses produced an improved ILS a greater ILS was obtained by the use in optimal dosage, of VP16-213 alone.

Vincristine and Chloroquine

Vincristine also showed activity against the TLX5 lymphoma (Fig. 3) and produced a maximum ILS of 158% (2.5 mg/kg). In contrast to VP16-213 the addition of chloroquine to vincristine does not appear to be additive. At higher dosage of vincristine toxicity again appeared to be enhanced.

Discussion

VP16-213 is now entering phase III studies in combination with other chemotherapeutic agents for the management of several types of tumour [1]. Its exact mechanism of action is unclear but one way in which it may act is by causing single strand breaks in DNA [15, 18]. The concept of combining drugs which damage DNA with compounds which are known to inhibit DNA repair is not new and several such inhibitory compounds are known [2, 16]. Chloroquine is known to bind to nucleic acid and to inhibit DNA and RNA Polymerase [3–4, 20]. Michael and Williams [15] were able to inhibit repair of DNA damage produced in cultured rat liver cells with chloroquine. Gaudin et al [7–8] demonstrated that chloroquine enhanced the effect of alkylating agents and X rays on resistent plasmacytomas and melanoma and attributed this effect to inhibition of DNA repair.

The results presented here show that chloroquine alone has no significant antitumour activity against the TLX 5 lymphoma even when used in near toxic doses. In combination with low doses of VP16-213 chloroquine appears to enhance the effect of the cytotoxic agent thus producing increased survival in tumour bearing mice. When higher doses of VP16-213 are combined with chloroquine this effect is reduced presumably due to toxicity which manifests as reversible weight loss. The enhancement of the effect of low dose VP16-213 by chloroquine is maximal when the two drugs are administered simultaneously; however significant enhancement still occurs when chloroquine is administered 6 h prior to VP16-213. This latter observation probably reflects the known long half life of chloroquine [6]. When chloroquine is administered 6 h after VP16-213 no enhancement is seen. If enhancement of cell killing is due to inhibition of repair mechanisms this suggests that repair has already largely occurred by 6 h. Vincristine – a drug not known to damage DNA is also active against TLX 5 lymphoma. In contrast to VP16-213 when vincristine is used in low dosage no enhancement of effect was seen when in combination with chloroquine.

The findings presented here add indirect evidence to the suggestion that single strand DNA breaks may be responsible for the activity of VP16-213. It is possible though that other mechanisms may be

responsible for this observation for example there may be an alteration in the pharmacokinetics of VP16-213 either by delay in elimination or by the prevention of its conversion to non active metabolites.

If the doses of chloroquine used in these studies were extrapolated to use in man they would cause considerable toxicity. It should also be pointed out that enhancement of the effect of VP16-213 with chloroquine can be reproduced by using VP16-213 alone in optimal dosage. While useful as a tool to investigate the mechanism of action of VP16-213 this particular combination would not appear to have a future place in the clinic. Nevertheless investigation of the interaction of chemotheraputic agents thought to damage DNA with antagonists of DNA repair deserves greater attention.

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Accepted July, 1981