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Synergism Between Hyperthermia and Cyclophosphamide *In Vivo*: The Effect of Dose Fractionation

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Abstract—*The synergistic anti-tumor interaction between heat and cyclophosphamide (CP) was investigated. Synergism between local hyperthermia (42.5°C, 30 min) and CP was observed in mice bearing the Lewis lung carcinoma tumor in their hind leg. Local hyperthermia reduced the CP dose needed for cure or a specific tumor growth delay. CP was less effective when given in fractions. A fractionated dose regimen was more effective than CP alone when combined with heat. The thermal enhancement ratio was larger when CP was administered in more than three fractions. It is concluded that combined heat and CP chemotherapy of cancer may be clinically feasible.*

INTRODUCTION

EXPERIMENTAL and clinical evidence shows that hyperthermia alone, or in combination with radiation or drugs, has a selective inhibitory and destructive effect on cancer cells (for a detailed bibliography, see *Cancer Therapy by Hyperthermia and Radiation*, 1978). Elevated temperatures (40–45°C) potentiate the response of mammalian cells to radiation [2–5] and cytotoxic drugs [5–7].

Recently, we have shown that heat enhances the inhibitory effect of cyclophosphamide (CP) on tumors inoculated into mice [8]. CP is commonly used in cancer chemotherapy [9]. However, as administered it has no activity as an alkylating agent; the hepatic mixed-function oxidases apparently produce 4-hydroxy CP as the initial oxidation product [10], which decomposes spontaneously to produce the active species, phosphoramide mustard [11, 12].

Relatively little has been reported concerning regimens of fractionated hyperthermia and radiation [13–16]. There is even less data on fractionation of hyperthermia and cytotoxic drugs as a treatment mode [17]. The aim of the present study was to investigate the effect of fractionated combined heat and CP treatment. The results suggest that the combined modality may be applicable to the clinical situation

MATERIALS AND METHODS

BDF mice, 5–6 months old, were used in these experiments. The tumor was the Lewis lung carcinoma (3LL). This malignant metastasizing tumor originated spontaneously in a C57BL/6 mouse [18]. The tumor was maintained in BDF mice as previously described [19]. Cell suspensions, prepared by mincing the tumor tissue, followed by gentle abrasion against a stainless steel screen, were filtered through a fine stainless steel mesh. Cells were counted in a hemocytometer and 3×10^5 viable cells (estimated by trypan blue exclusion) in 0.3 ml were injected i.m. into the left hind leg of the experimental animals. Tumor size was measured with a caliper. Two measurements were taken, one parallel and the other perpendicular to the animal's back, and the average of the two was used for estimation of tumor growth delay.

Local heating was applied using a thermostatted water bath at a temperature of 42.5°C. Animals were laid on PVC support with the tumor-bearing leg immersed in water through a hole of 12 mm diameter for 30 min treatment time. A fan was used to maintain body

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temperature at 36–37°C during heating (measured by a thermistor inserted under the back skin). Temperature measurements and control were as previously described [20]

*Table 1. The effect of CP dose and number of fractions on tumor growth delay with or without heat treatment**

Cyclophosphamide (mg/kg weight)	Heat treatment	Number of fractions	Tumor growth delay (days)
140	–	1	12
140	+	1	95% cure
70	–	2	12
70	+	2	100% cure
50	–	3	9
35	–	4	5
35	+	4	19
50	–	2	6
50	+	2	15
35	–	3	3
35	+	3	9
70	–	1	5
70	+	1	8
35	–	2	2
35	+	2	6
25	–	3	1
25	+	3	3
35	–	1	1
35	+	1	3
17	–	2	1
17	+	2	2

*CP was injected i.p. immediately before local heating (42.5°C, 30 min). Treatments started 3 days after tumor inoculation, and were administered every other day.

Experimental schedule

Animals were randomly divided into the following experimental groups. (1) Controls inoculated with tumor cells only; (2) i.p. drug-injected; (3) i.p. drug-injected immediately before heating and (4) local heating only. Treatments were started 3 days after tumor inoculation, and were given every other day. The number of treatments in each group is specified in Table 1. All animals, including controls, were anesthetized during treatment by an i.p. injection of pentobarbital sodium. This permitted positioning of the limb in the heating bath without mechanical fixing, thus avoiding impairment of blood supply to the limb.

Cyclophosphamide (cytophosphan-Taro) was obtained from Taro Pharm. Indust., Haifa, Israel. A single batch of drug was used to ensure uniform results. The drug was dissolved in saline and diluted to the required concentrations.

RESULTS

Previous data [8] indicated that local preheating immediately before drug injection had no advantage over administration of the drug alone. The present treatment schedule was selected since local heating (42.5°C, 30 min) immediately after CP injection was significantly superior to local heating 30 min after drug injection.

Table 1 summarizes tumor growth delay following different fractionation regimens of CP alone, or CP combined with heat. It should be noted that heat treatment by itself up to four treatments did not affect tumor growth. Figure 1 presents data obtained with

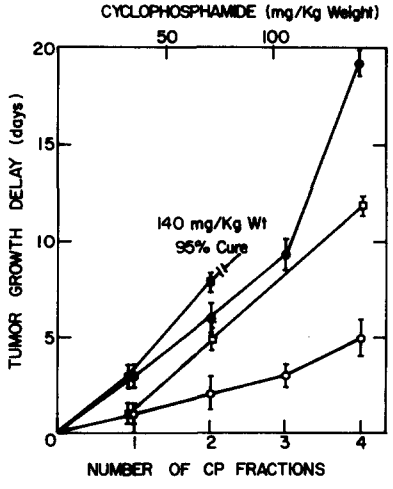

Fig. 1. Effect of normothermic and hyperthermic single and fractionated CP dose treatments on tumor growth delay. (●) CP (35 mg/kg) was injected i.p. immediately before local heating of the tumor (42.5°C, 30 min); (○) drug only; (■) single CP dose with, or without (□) local tumor hyperthermia (42.5°C, 30 min). Treatments started 3 days after tumor inoculation, and were administered every other day.

normothermic and hyperthermic single or fractionated dose treatments. In the drug concentration range tested, combined CP and heat resulted in tumor growth delay three to four times greater than that produced by the drug alone. Figure 2 summarizes the percentage cure of tumor-bearing animals treated with a single dose of CP with or without heat treatment. Cure is obtained with a single CP dose of 170 mg/kg (an animal is defined as cured when free of tumor 3 months after treatment). The TCD_{50} is reduced to 100 mg/kg when CP was followed by heat, corresponding to a thermal enhancement ratio (TER) of 1.7 as defined by Robinson *et al.* [21]. However, when tumor growth delay is chosen as an end point, a somewhat lower TER of 1.5 is obtained.

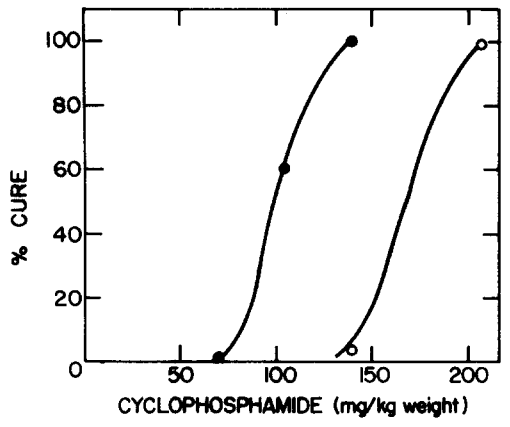

Fig. 2. Percentage of cure of tumor bearing animals treated with single doses of CP with (●) or without (○) local hyperthermia (42.5°C, 30 min).

DISCUSSION

We have demonstrated *in vivo* synergistic interaction of CP and local hyperthermia (42.5°C, 30 min) applied after CP injection [8]. Since local preheating offered no advantage over drug treatment alone, this investigation concentrated on fractionated heat post drug administration. Previous findings and the results of this study indicate that the *in vivo* synergistic interaction of CP+heat could be explained by enhanced uptake of CP by the heated tumor cells, and/or enhanced CP damage due to its greater reactivity at higher temperatures (for review of thermotherapy, see Hahn [22]). Fractionation by itself reduced the effectiveness of CP treatment (Table 1). However, when combined with local hyperthermia, synergism is observed (Fig. 1). Moreover, the TER is about twice as large as with a single CP treatment (Fig. 2). The reasons for this are not clear at present. Hahn *et al.* achieved a more pronounced effect than we found in this study probably due to differences in the effectiveness of treatment, attributable to either the lower CP dose in our study, or to the higher proliferation rate of the 3LL tumor, or both. It is noteworthy that when the number of fractions increases from three to four, the synergism of CP with heat is markedly enhanced (Fig. 1). A higher TER for drug plus heat treatment administered more than once was also reported by Marmor [17]. It is yet to be seen whether this holds true for a larger number of fractions. This might be of clinical importance, since about eight fractions are administered in clinical chemotherapy.

In conclusion, we have shown that local hyperthermia enhances the efficiency of CP in tumor control, and that the fractionated CP regimen was more effective when combined with local hyperthermia. These results indicate that when combined single or fractionated modality is more effective than drug alone, and may be so in the clinical situation.

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