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Thermal Enhancement of Bleomycin-induced Tumour Growth Delay: the Effect of Dose Fractionation*

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Abstract—*The influence of hyperthermia on bleomycin-induced growth delay of a syngeneic murine carcinoma has been studied in both simultaneous and sequential schedules for single and multi-fraction drug dose. The results reveal thermal potentiation of drug-induced growth delay if treatment is given simultaneously. This observed potentiation is independent of single or multi-fraction drug administration. Sequential pre-heating treatment resulted in only additive growth rate depression for single and two-fraction drug doses. However, when the drug dose was given in three fractions a significant thermal potentiation was observed for both simultaneous and sequential schedules.*

IN A recent paper [1] the authors reported potentiation of bleomycin-induced growth delay of a squamous cell carcinoma in CBA/Ht mice using a single dose of bleomycin (25 or 50 mg/kg) given concurrently with hyperthermia at 43°C for 15 min. If hyperthermic treatment were applied consecutively, even within 1 hr before or after drug administration, then only additive effects were observed.

Very few reports of the effects of fractionating the cytotoxic drug treatment in combination with hyperthermia have appeared in the literature. Hazan *et al.* [2] reported enhanced growth delay of a Lewis lung carcinoma by fractionation of cyclophosphamide treatment relative to one comparable dose when given in combination with local hyperthermia.

In view of the relevance of fractionation of drug treatment to the clinical situation, particularly in relation to undesirable side-effects from a large single dose, we have examined fractionation of the bleomycin treatment of the squamous cell carcinoma studied previously, in combination with hyperthermia.

The methodology of experiments described in this paper is identical to that reported previously [1]. The isogenous tumour was implanted intradermally or subcutaneously between the front and rear limbs of inbred CBA/Ht mice, the strain in which the tumour arose spontaneously. The approximately spherical tumours were palpable 6–7 days after implantation. Tumour growth delay, assessed by Vernier caliper measurement of tumour size in three dimensions, represents the extra time taken to reach a tumour diameter of 15 mm compared to controls. Initial tumour diameters were normalised to control values. All animals, including control groups, were anaesthetised during treatment by an i.p. injection of sagatal (sodium pentabarbitone) at a dose of 80 mg/kg in 0.2 ml before local heating.

Bleomycin effects at three drug concentrations were studied, 25, 50 and 75 mg/kg, in combination with ultrasound-induced local hyperthermia at 43°C for 15 min. The LD_{50} for bleomycin administered to this strain of mouse is approximately 200 mg/kg. The thermotherapy conditions were as follows: 45 sec at 1.5 W/cm^2, 0.75 MHz to raise the temperature of the tumour to 43°C. This temperature is maintained for a period of 15 min by insonating at 0.5 W/cm^2. Temperature profiles were obtained by insertion of the tip (0.8 mm) of a hypodermic thermocouple attached to a Digitron 175-K digital thermometer at different depths and positions in the tumour. At least six readings per tumour were taken. The initial temperature in the tumour of the anaesthetised animal of approximately 34°C rose to 43°C during the first 45 sec at 1.5 W/cm^2 and

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remained at this value ± 0.2°C during the period of subsequent exposure at 0.5 W/cm². The variation reflects the difference in temperature dependent on the depth at which the reading was taken. Simultaneous treatment implies heat application 20–30 min after i.p. injection of bleomycin. Previous results reported in [1] for this strain of mouse suggest maximum tumour/blood ratios of bleomycin are being achieved at that time, a finding in keeping within the results reported in [3] for Swiss mice. Pre-heating treatment implies hyperthermia immediately before bleomycin injection, separated by 30–45 min from the time of maximum tumour/blood bleomycin ratios.

Fractionated drug doses were given on successive days, 24 hr apart, with hyperthermia at 43°C for 15 min delivered simultaneously or pre-drug injection on each day.

$$\text{Dose-modifying factor (DMF)} = \frac{\text{growth delay for combined treatment}}{\text{growth delay for drug + growth delay for H/T}}.$$

The results in Table 1 indicate that growth delay is greater after simultaneous treatment with a single dose at any of the three drug doses studied when compared to pre-heating procedures. The fractionation regimes, involving two or three fractions of bleomycin 25 mg/kg, enhance tumour growth delay only marginally at the most, compared to the value obtained from the same total dose in one fraction for simultaneous treatment.

However, fractionation of drug dose in pre-heating experiments leads to a marked increase in tumour growth delay when this measurement is assessed for a single dose of 75 mg/kg bleomycin compared to 3 × 25 mg/kg given on successive days. It will be observed that two or three applications of hyperthermia alone given at 24-hr intervals have no effect on tumour growth delay compared to a single exposure.

A similar effect for hyperthermia alone was observed by Hazan *et al.* [2] for treatments of up to four fractions to Lewis lung carcinoma-bearing mice, compared to a single fraction. More recent, as yet unpublished data (Mahdi and Chapman, unpublished data), using the same procedures described in this paper, revealed heat-induced tumour growth delay of 4.1 days for one fraction (43°C for 15 min), 4.8 days for two fractions, 4.8 days for three fractions and 5.1 days for four fractions, collaborating the findings in this paper for heat treatment applied at 24-hr intervals. Increasing the time between fractions to 48 hr revealed enhanced effects of heat fractions compared to single fractions, suggesting that the heat-alone effects described here may possibly be attributed to thermotolerance.

These findings are reflected in the dose-modifying factors reported in Table 2. DMF

Table 1. *Tumour growth delay produced by single and multi-fraction regimens for therapy agents alone and in combination*

Total drug dose (mg/kg)	Hyperthermia at 43°C for 15 min	No. of fractions	Tumour growth delay (days) Single agent Bleomycin	Single agent Hyperthermia	Combined therapy Pre-heat	Combined therapy Simultaneous
25	+	1		3.6	6.0	10.5
	-	1	2.2			
50	+	1		3.6	8.4	15.2
	-	1	4.4			
	+	2		3.6	8.0	15.0
	-	2	5.8			
75	+	1		3.6	9.0	18.5
	-	1	5.6			
	+	3		3.6	19.0	23.0
	-	3	8.2			

Mean values based on 24 controls, 24 H/T only and 8 animals in each of the other groups.

Table 2. *Dose-modifying factors for single and multi-fraction regimens*

Total drug dose (mg/kg)	No. of fractions	Dose-modifying factor (DMF) Pre-heat	Dose-modifying factor (DMF) Simultaneous
25	1	1.0	1.8
50	1	1.05	1.9
	2	0.85	1.6
75	1	0.98	2.0
	3	1.6	1.95

values of greater than unity were recorded for simultaneous treatments irrespective of schedule, indicating thermal enhancement throughout. However, only additive effects are recorded in the pre-heating experiments for single or two-fraction regimes. When the drug dose is given in three fractions a DMF value significantly above unity is recorded, indicating potentiation.

Potentiation of the cytotoxicity of bleomycin administered s.c. 15 mg/kg immediately prior to tumour heating (43°C for 60 min) on days 4, 7 and 10 following tumour implantation was reported by Magin *et al.* [4], studying Lewis lung carcinoma. If the two treatments were given either 4 or 24 hr apart only an additive effect on growth delay was observed, emphasising the importance of scheduling and, in particular, the value of combined simultaneous treatment.

Reports both by Marmor *et al.* [5] and by Von Szczepanski and Trott [6] indicate that under certain scheduling conditions potentiation of bleomycin activity by hyperthermia is observed, measured by tumour growth delay or the development of metastases. In the former case the effect was only observed if the three fractions were administered simultaneously. No tumour cures were recorded if bleomycin and heat were given 24 hr apart. In the latter case the effectiveness of the combined treatment was much greater if bleomycin was administered before heating rather than after heating. Bleomycin was administered to C_3H mice 30 min before heating. Hence, from comments on tumour/blood ratios made earlier, this may represent simultaneous exposure to both agents.

The results reported in Table 2 in this paper for single-fraction pre-heating experiments indicate that, at best, only additive effects are achieved. The observation that potentiation may be achieved in multifraction treatment even if not given concurrently with hyperthermia is of interest in relation to clinical considerations, where multifraction simultaneous treatments may not always be practically feasible.

The mechanisms underlying the increased effectiveness of multifraction regimens are not clear. It has been shown [1] that hyperthermia does not enhance bleomycin uptake into cells, nor does it enhance bleomycin-induced DNA damage [7], though some effect of hyperthermia on repair of bleomycin-induced DNA damage may be observed. A likely mechanism contributing to potentiation may be hyperthermia-induced enhancement of bleomycin intercalation into DNA, as reported by Chapman *et al.* [8]. However, whilst these explanations may contribute to knowledge of potentiation in simultaneous schedules, single and multifraction, the reasons for the dose-modifying effects of multifraction regimens for sequential schedules (Table 2) compared to a single fraction have yet to be explained. A similar sharp threshold for effectiveness of a multifraction drug schedule, in combination with hyperthermia, is evident in the work of Hazan *et al.* [12]. They showed a dramatic increase in thermal enhancement of tumour growth delay by cyclophosphamide when the number of fractions was increased from three to four.

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