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The Combined Effect of Bleomycin and Hyperthermia on the Adenocarcinoma 284 of the C3H Mouse

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Abstract—*Isogeneic adenocarcinomas of female C3H mice were treated with bleomycin and local hyperthermia and tumour regrowth delay was measured. Hyperthermia had a synergistic effect on the drug induced growth delay. Heating to 44.5°C for 20 min, 30 min after bleomycin, increased the slope of the dose response curve by a factor of 2.7 whereas the same heat treatment given 30 min before the drug increased the slope of the dose response curve only by a factor of 1.5.*

INTRODUCTION

NUMEROUS experimental and clinical observations have recently demonstrated significant potentiation of the effectiveness of irradiation in murine and human tumours by the simultaneous or sequential applications of hyperthermia (e.g.[1–5]). Much less emphasis has been given to the study of possible interaction between hyperthermia and cytostatic drugs in tumours[6]. Experiments reported by Hahn[7] in chinese hamster cells *in vitro* suggested that especially the action of bleomycin might be enhanced by hyperthermia. The experiments reported here demonstrate that also in a solid tumour system studied *in situ* hyperthermia has a sensitizing effect on bleomycin.

MATERIAL AND METHODS

Experiments were performed on the isogeneic adenocarcinoma 284 transplanted subcutaneously into the flank of 8 weeks old females of the inbred Neuherberg strain of C3H mice. The growth characteristics of this tumour have been previously published in detail[8]. One week after transplantation the tumours became palpable. Subsequently, tumour diameters were measured 3 times per week with a perspex stencil containing holes of graded diameters increasing in steps of 0.3 mm. For every tumour, individual growth curves were plotted and the times to grow to various diameters were read directly from each curve. To draw the average growth curve of a group of 10 untreated or treated tumours, the geometric means of these times and their standard errors were calculated.

Tumours were treated when they had reached a diameter of 9 mm. The treatment effect was quantified by calculating the median time to grow from treatment size to a diameter of 13 mm. This criterion was introduced by Thomlinson and Craddock[9] into tumour radiobiology and has been used in subsequent studies by many authors. The initial volume changes after treatment were very variable and are not shown in Figs. 2–4, where only the regrowth curves are plotted since they contain all relevant information.

Hyperthermia was applied by completely immersing the tumour for 20 min into a water bath of 44.5 ± 0.1 or 43.5 ± 0.1°C. The technique was the same as in the studies on the combination with irradiation[3]. Care was taken to avoid obstruction of blood flow. Animals were anesthesized with 200 mg/kg hexobarbital before the application of hyperthermia. Tumour core temperatures measured with a thermocouple were found to be 43.5 and 42.5°C respectively, with little variability from tumour to tumour. Temperature uniformity within the tumour was ±0.3 and thus not worse than in the intramuscular tumours reported by Bleehen *et al.*[10]

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Bleomycin was diluted with saline and injected intraperitoneally in a volume of 0.1 to 0.5 ml either 30 minutes before the start of hyperthermia or 30 minutes after the end of hyperthermia. Single doses of 25, 50, 75 or 100 mg/kg were given.

After treatment, tumour sizes were measured daily and plotted as described. No animals were lost from illness in the observation period.

RESULTS

Hyperthermia alone had a significant effect on tumour growth (Fig. 1). The median tumour diameter of the untreated tumour increased linearly by 1.2 mm/day. Growth after hyperthermia was delayed but later proceeded at a similar rate as in the untreated tumour. Growth delay after 20 min at 43.5°C was 1.5 (± 0.3) days; after 20 min at 44.5°C it was 3.8 (± 0.3) days.

Bleomycin also imposed a dose-dependent growth delay. 100 mg/kg was about the maximal tolerable dose which already produced severe side effects. Animals lost weight and the hair coat became coarse after 3–4 days, but all animals gradually recovered after 8–10 days. After 75 mg/kg side effects were much less pronounced and 50 mg/kg has no obvious side effects. Tumour growth curves after bleomycin (Fig. 2) were not only displaced to later times, but the regrowth rate became slower with increasing dose. The time to grow from 11 mm to 13 mm was 1.4 days in the controls, increasing to over 2 days after 25, 50 and 75 mg/kg and 2.5 days after 100 mg/kg. The time to grow to 13 min increased with increasing dose.

Bleomycin given 30 min after hyperthermia of 44.5°C further increased the growth delay (Fig. 3). As in unheated tumours, the growth rate

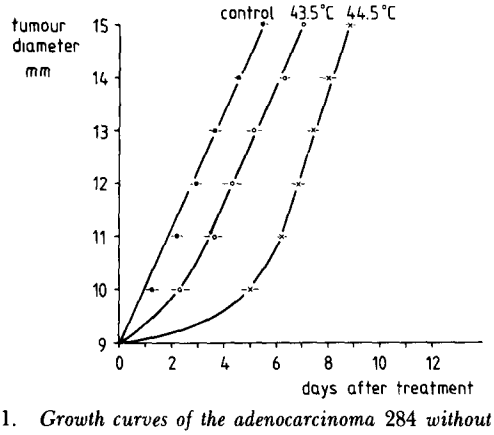

Fig. 1. Growth curves of the adenocarcinoma 284 without and after local heating in a waterbath of 43.5° or 44.5°C for 20 min. The points are the geometric means of the times the tumours needed to grow from a diameter of 9 mm to the respective diameters and their standard errors.

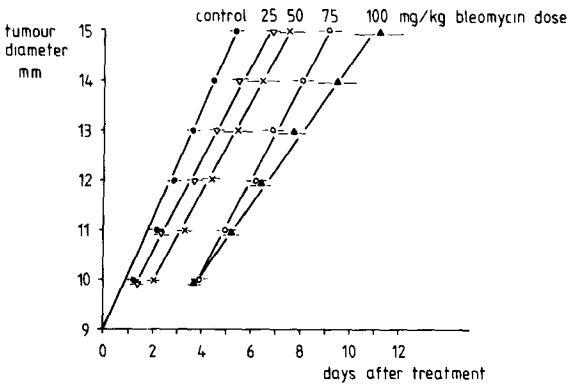

Fig. 2. Growth curves of the adenocarcinoma 284 treated with various doses of bleomycin. The data are plotted the same way as in Fig. 1.

decreased after bleomycin from 1.3 days for the growth from 11 mm to 13 mm in control animals to between 3 and 3.5 days for the same increase in diameter, differences between the various doses of bleomycin not being significant. Yet additional regrowth delay, i.e. the time after treatment to reach 13 mm diameter with the delay caused by heat alone subtracted, increased with bleomycin dose.

Hyperthermia given 30 min after bleomycin had much greater effect on tumour growth than the reversed sequence (Fig. 4). As in the other experiments the growth rate after bleomycin decreased compared to the tumours heated only but this effect was independent of bleomycin dose. The additional regrowth delay caused by the bleomycin given before hyperthermia increased much more than in the experiment shown in Fig. 3 (results plotted in Fig. 5). Even heating the tumour to only 43.5°C increased the action of bleomycin more if the drug was given first than heating to 44.5°C with subsequent drug injection (Fig. 5).

In Fig. 5 the various response curves of the action of bleomycin without or in combination with hyperthermia are summarized, showing

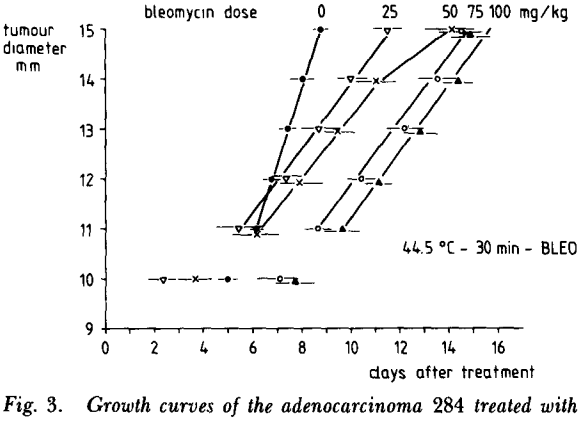

Fig. 3. Growth curves of the adenocarcinoma 284 treated with 44.5°C hyperthermia followed 30 min later by various doses of bleomycin. The data are plotted the same way as in Fig. 1.

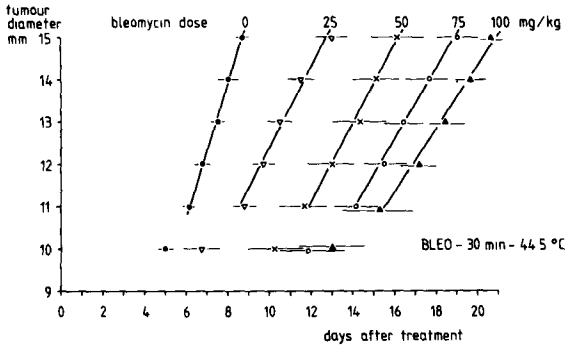

Fig. 4. Growth curves of the adenocarcinoma 284 treated with various doses of bleomycin followed 30 min later by 44.5°C hyperthermia. The data are plotted the same way as in Fig. 1.

the dependence of the additional delay to grow to 13 mm diameter, with the delay caused by heat alone subtracted, on bleomycin dose. All curves could be approximated by a linear relationship between additional delay and bleomycin dose. The effectiveness of the various combinations can therefore be compared by calculating slopes or the doses to reach a given effect (e.g. 4 days additional regrowth delay). 44.5°C hyperthermia given before the drug increases the slope by a dose modification factor of about 1.5, 43.5°C hyperthermia given after the drug by a DMF of about 1.8 and 44.5°C hyperthermia given after the drug by a DFM of 2.7.

DISCUSSION

The results of our experiments show that hyperthermia given before or after bleomycin increased the slope of the drug dose–response curves and thus interacted with the drug in a synergistic mode. They are in accordance with *in vitro* observations on the combination of bleomycin and hyperthermia in various cell lines. Braun and Hahn[11] observed maximal sensitization of Chinese hamster cells (HA 1) at temperatures above 41°C when heating and drug exposures were simultaneous. Hyperthermia did not increase drug uptake into the cells, as was also observed by Hassanzadeh and Chapman[12] in HeLa cells. In addition, the sequence of heating and drug exposure had a great influence on cell survival. Preheating immediately before drug exposure at 37°C decreased the surviving fraction much more than post-heating, yet repair of potentially lethal damage was only inhibited by post-heating. Rabbani *et al.*[13] observed pronounced sensitization of HeLa cells to bleomycin by 42°C hyperthermia if both treatments were given simultaneously. Hassanzadeh and Chapman[12], too, found the most pronounced sensitization of HeLa cells with simultaneous treatment and only insignificant sensitization if 42.5°C hyperthermia was given 1 hr after bleomycin exposure. While the slope of the cell survival curve increased nearly five-fold by the simultaneous heating to 43.5°C, the frequency of DNA strand breaks after bleomycin exposure was the same with and without hyperthermia[12].

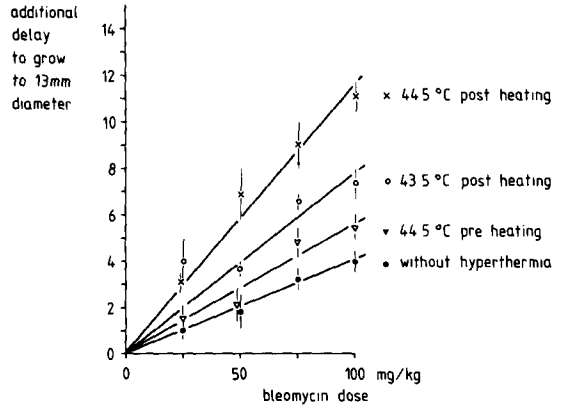

Fig. 5. Dose–response relationships of additional tumour regrowth delay on bleomycin dose for various schedules of combined drug—hyperthermia treatment. Additional regrowth delay is the total time to reach a diameter of 13 mm minus the time the tumours only treated by hyperthermia needed to reach 13 mm.

The combination of bleomycin and hyperthermia in an experimental tumour was studied by Marmor[14] in the KHT sarcoma. Sensitization was observed only at temperatures above 42°C and only if both treatment modalities were given simultaneously. Probably as a result of its immunogenicity, even tumour cures were registered in the KHT sarcoma after 2 treatments with 15 mg/kg bleomycin during 43°C hyperthermia. Our experiments enlarge the observations of Marmor on an isogeneic tumour, a larger spectrum of drug doses, temperatures, and schedules but essentially confirm their conclusions. It appeared that hyperthermia exerts its maximal effect when given shortly after drug injection, i.e. at the time of peak drug concentration in the blood. The action of hyperthermia appears to be clearly dose-modifying: e.g., a drug dose of 25 mg/kg followed 30 min later by 44.5°C hyperthermia for 20 min has the same effect as 75 mg/kg without hyperthermia. This dose-modifying action is temperature dependent and is smaller if applied before the drug is given.

The mechanism for the interaction of hyperthermia and bleomycin remains obscure. Since hyperthermia has a profound effect on membrane permeability[15] it would be an attractive interpretation to assume increased

uptake of the drug, especially since maximal effectiveness was found at the time of peak serum concentration. However, the *in vitro* studies [11,12] measured lower cellular uptake of radioactively labelled bleomycin rather than increased uptake. Inhibition of PLD-repair may explain at least part of the increased effectiveness of post-heating as compared to pre-heating. Yet at the present stage of knowledge further speculations on the possible mechanisms seem premature.

The reported data suggest a great potential for the combined application of extreme local hyperthermia and bleomycin in the local treatment of selected tumours. Clinical observations reported by Arcangeli *et al.*[16] on node metastases of squamous cell carcinomas showed a markedly improved tumour response after bleomycin given twice daily for 10 days to a total dose of 150 mg when the tumours were treated between both daily bleomycin doses to over 42°C. Hyperthermia increased the response rate from 3/6 to 7/7 and produced lasting remission for over 4 months in 2/5 patients.

With better knowledge of the mechanisms involved and the influence of scheduling and dosage those results may be improved even further.

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