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Combined Adriamycin and Hyperthermia Treatment of a Murine Mammary Carcinoma *in Vivo*[1]

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SUMMARY

A study was made of the effect of combined adriamycin and hyperthermic treatment in a solid mouse mammary carcinoma *in vivo*.

This study demonstrated: (*a*) that, when given separately, adriamycin and hyperthermia enhance the destruction of a solid mouse mammary carcinoma *in vivo*; hyperthermia alone in high doses may even cause long-time survival; (*b*) that the combination of adriamycin and local hyperthermia (40.5-42.5°) greatly increases tumor destruction and, in a number of cases, causes initial and long-time regression; (*c*) that whole-body hyperthermia in combination with adriamycin gives a significant delay in tumor growth as compared with the controls, but not to the same degree as the local combined therapy; and (*d*) that treatment with local hyperthermia and adriamycin gives a pronounced decrease in the lethal toxicity of adriamycin.

The effect of adriamycin and heat treatment may be due to hyperthermic cell destruction in the central area of the solid tumor, together with a synergistic effect of heat and adriamycin on the proliferating peripheral tumor cells.

Furthermore, local heat application may increase the adriamycin concentration in the heated tumor area, which causes a high destructive effect and a less toxic influence on the nonheated normal tissue.

INTRODUCTION

Recent studies have confirmed and extended the old observation that heat may cause complete and selective tumor destruction of malignant cells (6, 8, 22, 23, 29, 34).

Even though all tumor cells may be destroyed, hyperthermia characteristicly seems to be most pronounced in the nonproliferating tumor cells situated in the central area of solid tumors *in vivo* (17, 27, 37). These cells are normally relatively resistant to clinical therapy, and it may therefore be of value to combine hyperthermic treatment with other modalities that have a special action on the proliferating tumor population in the periphery of solid tumors.

For these reasons, there has been growing interest in the use of hyperthermia in combination therapy, first of all, with radiotherapy (15, 25, 30-32, 35, 36).

Heat is also known to increase the influence of various cytotoxic agents (4, 5, 13, 21, 25, 33).

Among recent studies of such "thermochemotherapy" are the observations by Hahn *et al*. (16, 18) that the effect of adriamycin increases when cells *in vitro* are exposed to the drug under hyperthermic conditions at 42-43°. Also tumor cells subjected *in vivo* to combined adriamycin and hyperthermic treatment showed a poorer *in vitro* survival, compared with cells treated *in vivo* with adriamycin only. In their study, Hahn *et al*. (18) investigated mainly the effect of combined heat-adriamycin treatment *in vitro*, and no experimental clinical observations were made.

The aim of the study reported here was to investigate the clinical effect of combined adriamycin and hyperthermic treatment on a murine mammary carcinoma *in vivo*.

MATERIALS AND METHODS

Animal and Tumor. One hundred fifty female and male C3D2F$_1$/BOM mice [C3H × DBA/2 F$_1$ (hereafter called C3D2F$_1$)] about 6 to 8 weeks old, were inoculated into the flank with an isologous, poorly differentiated mammary carcinoma which had arisen in the C3D2F$_1$/BOM strain. After inoculation with a volume of 15 μl tumor suspension, more than 95% of the mice showed positive local tumor growth which, in all cases, resulted in death of the animals within 5 weeks after transplantation. Metastases were late and rare.

Treatment. Eight- to 10-day-old tumors with a volume of about 100 cu mm were distributed randomly to one of the following groups: (*a*) no treatment (control group); (*b*) local hyperthermia (40.5°, 120 min); (*c*) adriamycin (25 mg/kg i.p.) plus local hyperthermia (40.5°, 120 min); (*d*) local hyperthermia (42.5°, 60 min); (*e*) adriamycin (25 mg/kg i.p.) plus local hyperthermia (42.5°, 60 min); (*f*) adriamycin alone (25 mg/kg i.p.); (*g*) whole-body hyperthermia (40.5°, 120 min); and (*h*) adriamycin (25 mg/kg i.p.) plus whole-body hyperthermia (40.5°, 120 min).

In each group, 10 to 20 mice were exposed to treatment, but animals that died during or immediately after the hyperthermic treatment were not included in the evaluation.

Adriamycin (NSC 123127) (10) (Adriablastina; Farmitalia, Milan, Italy) was given as a single i.p. injection in a dose of 25 mg/kg.

In the groups of mice to which adriamycin was given in a combined regimen, the injection was given i.p. 5 min before heating.

Local hyperthermia was performed by shortwave diathermia by means of the technique previously described (29), and following anesthesia with sodium pentobarbitate (Nembutal; Abbott Laboratories, Chicago, Ill.) 70 mg/kg given i.p. The temperature in the tumor was continuously

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measured during the treatment with a special calibrated thermocouple (29). The temperature was maintained within a range of ±0.1°.

Whole-body hyperthermia was given with the anesthetized mice placed in an isolated copper box covered with a glass plate. The box was heated by an infrared lamp through the glass, and the mice were cooled and ventilated by periodic air infusion. Temperature measurement was made continuously with a thermocouple in the tumor during the treatment. The interval from the start of treatment until the desired temperature was obtained (about 20 min) was not included in the treatment time.

Evaluation of Results. After treatment, the tumors were measured on unanesthetized animals at least 3 times a week with a slide gauge. The volume of the globular or ellipsoid tumors was calculated from the formula $(\pi/6) \times D_1 \times D_2 \times D_3$, where D is 3 orthogonal diameters (19). All animals that did not present complete tumor cure were observed until they either died from the tumor or were killed, with a tumor volume exceeding 10 ml. Animals in which tumor cure was obtained were observed for at least 120 days after treatment.

"Growth time" was defined as the period from treatment to the time when the tumors reach a volume of 5000 cu mm. Long-time survival was calculated 120 days after treatment.

RESULTS

Tumor Response

Hyperthermia Alone. Local treatment with 40.5° for 120 min caused a regression and delay in tumor growth (Chart 1), but although initially a single tumor showed complete regression, no permanent cure was obtained (Table 1).

Whole-body hyperthermic treatment with the same temperature and time (Chart 2) presented a less prominent delay in tumor growth, and no complete initial regression was seen (Table 2).

Local heating with a higher dose (42.5°, 60 min) resulted in regression and delay in tumor growth (Chart 3), which was not significantly different from that of local treatment with the lower local dose (Table 1). However, a higher degree of initial regression was found in this group, and long-time survival could be observed.

The increase in tumor growth occurring during the 1st day after treatment with local heat application was due to transient edema in the tumor area.

Adriamycin. Adriamycin given alone as a single i.p. injection of 25 mg/kg resulted, after some days' latency, in a significant delay in tumor growth (Charts 1 and 3), but no

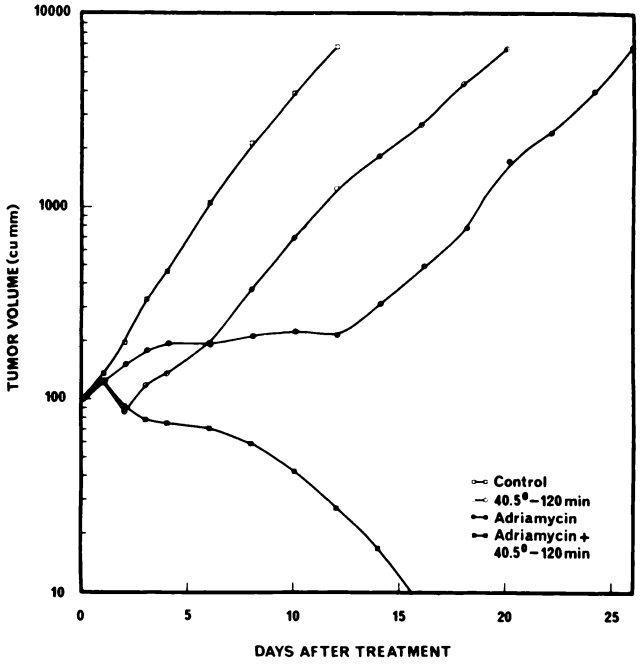

Chart 1. Effect of combined adriamycin (25 mg/kg) and low local hyperthermia (40.5°, 120 min) on the tumor volume of a solid murine mammary carcinoma *in vivo*. The effect is compared with untreated controls (tumors treated only with adriamycin or local hyperthermia).

Table 1

Effect of adriamycin and local hyperthermia in combined treatment of a solid mouse mammary carcinoma in vivo

Adriamycin dose (mg/kg i.p.)	Local hyperthermia (°/min)	No. of regressions[a]/no. treated		Median growth time (days)[b, c]	Increase in growth time[d] (%)	No. of cures/no. treated	No. of toxic deaths/no. treated
		Partial	Complete				
Controls		0/15	0/15	12.0		0/15	0/15
	40.5/120	5/10	1/10	17.5	45	0/10	
	42.5/60	4/10	2/10	17.0	42	1/10	
25		1/20	0/20	25.0	108	0/2[b]	18/20
25	40.5/120	3/14	7/14	40.6	238	3/7[b, e]	7/14[f]
25	42.5/60	4/13	7/13	>120.0	>900	5/8[b, g]	5/13[h]

[a] Partial, less than 50% reduction in original tumor mass; complete, regression to below palpable size.
[b] Mice that died from toxicity are excluded.
[c] Growth time to 5000 cu mm in volume.
[d] Increase in growth time: $\frac{\text{growth time of treated} - \text{growth time of controls}}{\text{growth time of controls}} \times 100\%$.
[e] Significantly different from controls, $p < 0.03$.
[f] Toxicity significantly lower than after adriamycin alone, $p < 0.002$.
[g] Significantly different from controls, $p < 0.002$.
[h] Toxicity significantly lower than after adriamycin alone, $p < 0.003$.

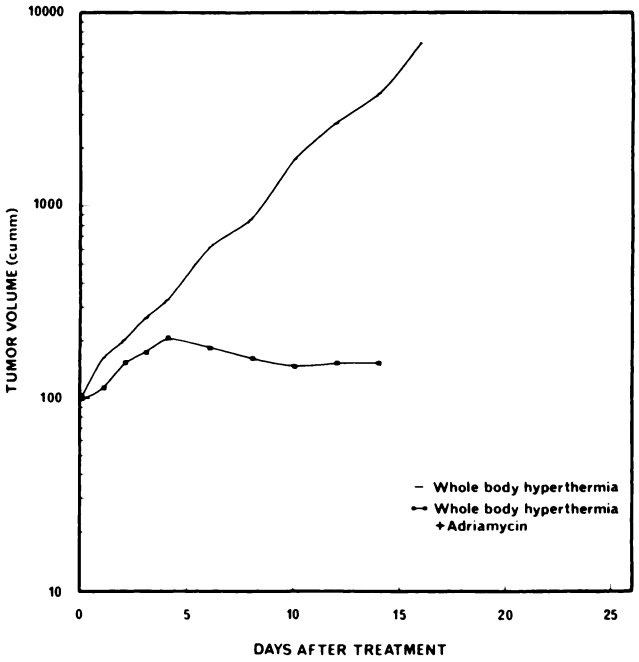

Chart 2. Effect of whole-body hyperthermia (40.5°, 120 min) alone and in combination with adriamycin (25 mg/kg). In the group treated with the combined regimen, all animals died within 15 days as a consequence of toxic symptoms.

tumors showed complete regression (Table 1).

Combined Treatment. Combined treatment with adriamycin and local hyperthermia resulted in a marked increase in tumor response. In both groups (40.5°, 120 min; and 42.5°, 60 min) initial regression with complete disappearance of the tumor was seen in most cases (Table 1). This disappearance occurred usually within 2 weeks after treatment (Charts 1 and 3). The time required for complete regression of the tumors initially after treatment was not significantly different in the 2 groups.

However, as assessed in terms of long-time survival, the frequency of recurrence was higher in the adriamycin-low local heat dose group than in the adriamycin-high local heat dose group (Table 1). However, in both groups, significantly better results were obtained than when adriamycin or local hyperthermia were administered alone (Table 1).

When adriamycin was given in combination with whole-body hyperthermia (40.5°, 120 min), the clinical response seemed to be less distinct than after combination with local hyperthermia. No initial or long-time regression was observed in this group, and the general impression was that this procedure was distinctly inferior to the combination of local heat treatment and adriamycin.

Toxicity

The dose of adriamycin used in this study was very high (25 mg/kg), and most of the animals receiving this treatment alone died from toxicity about a week later (Tables 1 and 2). A few days before death, their weight decreased, and the animals became weak and atonic with a tousled skin.

Given alone in a dose of 25 mg/kg body weight, adriamycin killed 90% of the mice. In contrast, only 7 of 14 and 5 of 13 of the animals treated with a combination of adriamycin and local hyperthermia showed lethal toxicity. It therefore seems that combination with local heat not only enhances the tumor response, but also decreases the toxicity of adriamycin (Table 1). However, when the animals were given combined adriamycin and whole-body hyperthermia, the

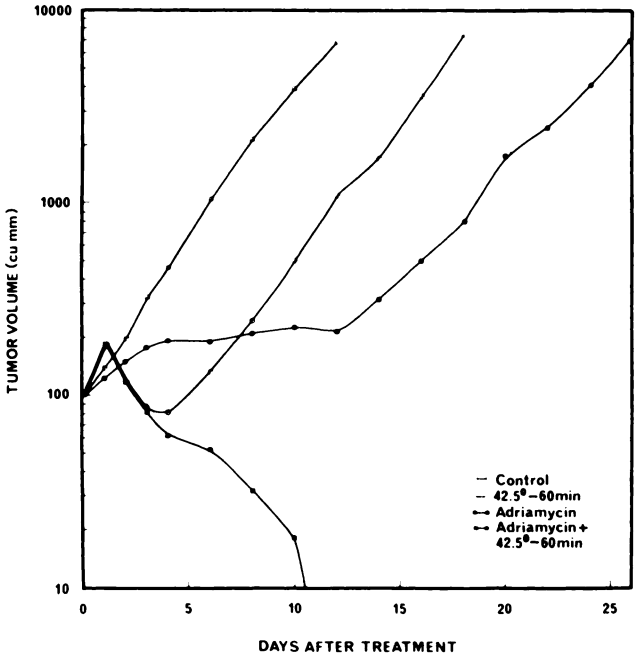

Chart 3. Effect of combined adriamycin (25 mg/kg) and high local hyperthermia (42.5°, 60 min) on the tumor volume of a solid murine mammary carcinoma *in vivo*. The effect is compared with untreated controls (tumors treated only with adriamycin or local hyperthermia).

Table 2

Effect of adriamycin and whole-body hyperthermia in combined treatment of a solid mouse mammary carcinoma in vivo

Adriamycin dose (mg/kg i.p.)	Hyperthermia (°/min)	No. of regressions[a]/no. treated		Median growth time (days)[b, c]	Increase in growth time (%)[d]	No of cures/no. treated	No. of toxic deaths/no. treated
		Partial	Complete				
Controls		0/15	0/15	12.0		0/15	0/15
	40.5/120	0/6	0/6	16.7	139	0/6	
25	40.5/120	2/7	2/7			0/0[b]	7/7

[a–d] See Table 1, Footnotes *a* to *d*.

toxic reaction seemed to be similar to that observed after administration of adriamycin alone (Table 2).

DISCUSSION

The present study showed that adriamycin given in combination with local hyperthermia increased the frequency of tumor destruction and cure in a solid mouse mammary carcinoma *in vivo* and that, in addition, the treatment decreased significantly the lethal toxicity following adriamycin treatment.

The effect of adriamycin and local hyperthermia given singly correspond to what has been obtained in other tumor systems in terms of tumor regression and growth (11, 14, 25, 29, 40).

The action of the adriamycin-hyperthermic effect on solid tumors *in vivo* seems to be a synergistic effect of heat and adriamycin on the proliferating cells in the periphery of the tumor tissue combined with a hyperthermic tumor cell destruction which is especially pronounced in the nonproliferating central area of the tumor.

The mechanism by which heat increases the effect of adriamycin may be an increased uptake of drug in the tumor cells combined with a synergistic effect of heat and drug on the cells.

That tumor cells increase the accumulation of adriamycin under hyperthermic conditions was shown *in vitro* by Hahn *et al*. (16, 18), and it seems also to be the case under *in vivo* conditions (Bichel and J. Overgaard, unpublished observations).

The sensitizing effect on proliferating tumor cells can be related to an enhanced inhibition of nucleic acid synthesis, as both modalities are known to have that effect (7-9, 38). An inhibited repair of DNA damage, which is known to be one of the factors in the heat-sensitizing effect of irradiation (2, 3) and bleomycin (5, 7), may also be a possible mechanism.

The enhanced tumor response of the combined treatment was also observed by Hahn *et al*. (18) in an *in vitro* investigation on Chinese hamster cells. However, in their *in vitro* system, the synergistic influence was present only in the range of about 43°, and no combined influence was observed at a lower temperature (41°). In the present *in vivo* study of a solid mammary carcinoma, also, heat at a lower temperature level (40.5°) in combination with adriamycin significantly increased the tumor cell destruction.

The reason for this difference in the effective temperature range may be related to the important fact that the hyperthermic response *eo ipso* is more intense in solid tumors treated *in vivo* than in similar cells kept under *in vitro* conditions (32). This increased sensitivity *in vivo* may be due mainly to the influence of the extracellular environment in the solid tumor tissue. Increased acidity and low oxygen tension in the extracellular space are factors that are known to increase the hyperthermic tumor cell destruction (12, 24, 26, 28). These changes may be most pronounced in the poorly vascularized central part of the solid tumor. In this area, most tumor cells are in a nonproliferating (G_0) state (39), which may further increase the effect, since density-inhibited plateau-phase cells, especially under unfed conditions, are observed to be much more sensitive to heat than exponentially growing tumor cells (17, 37).

The limitation of the use of adriamycin is especially related to the cardiac toxicity (42). Several attempts have been made to increase the adriamycin concentration selectively in malignant cells in order to reduce the toxic side effects. In particular, this has been done by using "lysosomotropic therapy" with special uptake in lysosomes of an adriamycin-DNA complex (1, 41) or, to a lesser extent, by binding adriamycin to antibodies (20), but none of these procedures seems to have given convincing results in solid tumors. The decreased toxicity observed in the animals treated with combined local hyperthermia and adriamycin is therefore a distinct and important feature. This effect may be related to the fact that local heating of a certain area increases the accumulation of the drug in the heated area and consequently decreases the accumulation and the toxic influence of the drug in the normal tissues. This view is supported by the observation that in combined treatment with whole-body hyperthermia the toxic effect on the animals seems similar to that observed after adriamycin given alone at a normal temperature.

In view of the results obtained in this animal study, it might be possible to use a combination of adriamycin and local hyperthermia in clinical practice on the treatment of local tumors. This therapy would, for example, be useful in the treatment of local recurrences in areas previously exposed to radiotherapy.

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