[CANCER RESEARCH 52, 487–490, January 15, 1992]

Advances in Brief

Interstitial Fluid Pressure in Solid Tumors following Hyperthermia: Possible Correlation with Therapeutic Response[1]

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Abstract

Elevated interstitial fluid pressure (IFP) of tumors may be a physiological barrier to the delivery of certain therapeutic agents. The objective of this study was to find out if IFP could be lowered using localized hyperthermia and if the reduction in IFP could predict the tumor response to treatment. Amelanotic melanoma (A-Mel-3) implanted into the dorsal skin of Syrian golden hamsters was exposed to hyperthermic treatment after 7 days of tumor growth at tumor volumes of about 100–150 mm³. Hyperthermia was induced by immersing the tumor in a water bath at 43°C for 30 or 60 min. Forty-eight h later the IFP of control and treated tumors was determined by using the wick-in-needle technique. The mean IFP in control tumors was 12.6 mmHg. Hyperthermic treatment for 30 min induced a significant decrease to 2.8 mmHg ($P < 0.001$ *versus* controls), whereas a 60-min immersion of the tumors induced a further decrease to 0.8 mmHg ($P < 0.05$ *versus* 43°C for 30 min). Separate experiments on tumor growth in corresponding groups of animals revealed a significant growth delay of 2.7 days after hyperthermia for 30 min. Enhanced growth delay and partial tumor response in 66% of the tumors were found following 60 min of hyperthermia at 43°C. The thermal dose-dependent decrease in IFP presumably results from the dose-dependent damage to the tumor vasculature. In addition, the association of an enhanced biological effect with a more pronounced reduction of interstitial fluid pressure suggests that the IFP might serve as a quantitative parameter to predict the response of tumors to hyperthermic therapy.

Introduction

Since the pioneering studies of Young *et al.* (1) in 1950 it is known that the IFP[3] which is nearly 0 mmHg in normal tissue, is significantly elevated in experimental tumors (2). Interstitial pressure may increase with growth in some tumors. Furthermore, in experimental tumors with a single nodule, IFP is relatively uniform and drops precipitously in the periphery at the tumor–normal tissue interface (3). Some of these findings have recently been confirmed in human tumors *in loco* (4–6). However, there is a general lack of information about changes in the IFP of tumors in response to therapy. Recently, Roh *et al.* (4) have shown that fractioned radiotherapy may lower IFP in carcinomas of uterine cervix in some patients, and this reduction in IFP may be useful in predicting treatment outcome. Whether this is true for other treatment modalities such as HT is not known. Since hyperthermia, similar to radiation, can impair tumor cells as well as tumor microcirculation, we wanted to find out if HT could lower IFP in tumors and if changes in IFP could represent a predictive marker for treatment response.

Materials and Methods

Animals and Tumor System. Our study was carried out using male inbred Syrian golden hamsters (9–11 weeks old, 90–110 g body weight). Cells ($4–6 \times 10^6$) of the amelanotic melanoma A-Mel-3 were suspended in a volume of 10 μl and implanted s.c. over the dorsal thorax and lumbosacral region. Therefore, animals were anesthesized i.p. with 50 mg/kg body weight pentobarbital. Seven days later tumors had reached a volume of 100–150 mm³. All hamsters developed tumors, and no spontaneous regression occurred.

Tumor Growth Evaluation. Tumor size was determined by calculation of tumor volume according to the formula

$$V_T = 0.873\ a{*}b{*}h$$

The longer (a) and the shorter (b) perpendicular axes and the height (h) of the tumor nodule were measured, and tumor weight was calculated assuming a specific tumor density of about 1 g/cm³ (7). The time to reach defined tumor volumes (0.25, 0.50, 1.00, and 2.00 cm³) was calculated for the individual tumors using an exponential growth function. Tumor response to treatment was classified as: complete response (the disappearance of all signs of a tumor); partial response (>50% reduction in the product of the largest two perpendicular tumor diameters for a minimum of 7 days); or no response (<50% reduction, or a decrease <25% in the product of the largest two perpendicular tumor diameters for a minimum of 5 days).

Hyperthermic Treatment. Hamsters were anesthetized, and a dorsal skin flap bearing the tumor was pulled through a small slit of a perspex tube isolated with polystrene. The skin flap was fixed to an arch with three sutures and submerged into a temperature-controlled water bath (Thermomix UB; B. Braun, Melsungen, Germany) at a temperature of 43.0 ± 0.01°C. During tumor heating the rectal temperature did not change.

Interstitial Fluid Pressure Measurements. During IFP measurements the mean arterial pressure was monitored continuously via a catheter (PE 10) implanted into the right carotid artery. IFP was measured by means of the WIN technique (8). A 23-gauge hypodermic needle (23G; Microlance, Dublin, Ireland) was prepared with a 2–3-mm side hole 3 mm from the tip of the needle. The edges were polished to provide a smooth surface against the hole. A multifilamentous nylon thread from a surgical suture (EH 6621; Ethicon, Nordersted, Germany) was placed within the needle. For pressure recording the needle was connected to a pressure transducer (DTXplus; SpectraMed, Düsseldorf, Germany) by polyethylene tubing (PE50) containing physiological saline. Signals of the mean arterial pressure and the interstitial fluid pressure were amplified (Pressure Amplifier 863; Siemens, Munich, Germany) and recorded on a dual-channel chart recorder (Kompensograph X-T; Siemens). The system was calibrated prior to and after each measurement by determining the linearity of the relationship between an imposed pressure signal induced by a Gauer mercury manometer and the meas-

Received 9/27/91; accepted 11/20/91.

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[1] Supported by a grant from the Bundesministerium für Forschung and Technologie (0706903A5) to A. E. G. and grants from the National Cancer Institute (CA37239 and CA49792) to R. K. J. M. L. is recipient of a Feodor-Lynen Fellowship (1991–1992), and R. K. J. was recipient of a Humboldt Senior Scientist Award (1990–1991).

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[3] The abbreviations used are: IFP, interstitial fluid pressure; WIN, wick in needle; HT, hyperthermia; MAB, monoclonal antibodies.

ured pressure. IFP measurements were performed by inserting the needles into tissue leaving them in place without external fixation. Needles were marked for exact placement in the tumor center and for localization of the site of measurement. Therefore the sensing area of the needle was always in the center of the tumor. As an internal control the interstitial fluid pressure was measured in the subcutaneous tissue of the tumor-free dorsal skin.

During all measurements, the body temperature of the hamsters was kept constant between 33°C and 35°C by a feedback-controlled heating pad. Room temperature was between 23°C and 25°C.

Experimental Procedure. The IFP in the tumors was determined 48 h after hyperthermic treatment. After system calibration and cannulation of the right carotid artery, the anesthetized hamsters were placed on the heating pad. Following zero pressure determination obtained by placing the needle in a saline drop placed on the tumor, the WIN needle was inserted into the center of the tumor. After 1–5 min the initially negative pressure declined, first rapidly and then more slowly and restabilized at the original level. Following this initial equilibration period, the interstitial fluid pressure was stable. At the end of each measurement, fluid communication between needle and interstitium was checked. Measurements were accepted only when satisfactory fluid communication between needle and tissue fluid could be demonstrated by compressing and decompressing the polyethylene tube with a screw clamp (8).

The tumor growth and the interstitial pressure of tumors were evaluated in separate series of experiments. The effect of local hyperthermia on tumor growth was quantified by measuring tumor diameters before and every second day after local hyperthermia at 43°C for 30 min ($n = 6$) or for 60 min ($n = 6$). Furthermore, tumor volume was determined in an untreated control group at the same time points of measurement ($n = 6$). In a second series of experiments the IFP of tumors was measured with the WIN, 48 h after local hyperthermia. In each animal the IFP was monitored in tumors treated by hyperthermia at 43°C for 30 min ($n = 14$) or for 60 min ($n = 14$), in sham-treated control tumors ($n = 9$) and in the subcutaneous skin tissue as an internal control.[4]

Statistics. Nonparametric one-way analysis of variance and multiple comparison on ranks of several independent samples were done using the Kruskal-Wallis test. Single comparisons of independent samples were performed using the Wilcoxon test and of related samples using the U test. P values smaller than 5% were considered significant. All results are represented as mean and median ± SE of the median.

Results

Growth curves of untreated control and tumors heated to 43.0°C for 30 or 60 min are presented in Fig. 1. The control tumors revealed an exponential tumor growth, whereas the growth of tumors receiving hyperthermia for 30 min was significantly inhibited for the first 6 days ($P < 0.01$), but then the tumors grew exponentially. The exponential part of the growth curves was nearly parallel. Hyperthermia with a duration of 60 min resulted in a marked delay of tumor growth in comparison to tumors treated for 30 min ($P < 0.01$) and in comparison to control tumors ($P < 0.001$). Table 1 demonstrates the calculated tumor growth times. Hyperthermia for 30 min induced a tumor growth delay of 2.7 days. Tumors treated with hyperthermia for 60 min did not reach a volume of 0.25 cm^3 for the whole period of observation. Thus tumor growth times and growth delay could not be calculated.

Table 2 demonstrates that a complete tumor response was observed in none of the experimental groups. Partial tumor response was seen in four of six animals treated at 43°C for 60 min. No response in tumor mass was seen in two of six tumors of both treatment groups, whereas all tumors of the control group showed a progressive volume increase.

Mean arterial pressure during IFP measurements (138 mmHg; median = 140, SE = 8.7) and IFP in the tumor-free skin (Fig. 2) did not vary significantly between the different treatment groups. Mean subcutaneous interstitial fluid pressure was −1.0 mmHg (median = −0.7, SE = 0.4, $n = 8$) in the control group, −1.7 mmHg (median = −2.0, SE = 0.1, $n = 9$) in the group treated with local hyperthermia for 30 min, and −1.5 mmHg (median = −1.1, SE = 0.3, $n = 10$) in animals treated with hyperthermia for 60 min.

Interstitial fluid pressure in tumors was significantly elevated compared to IFP in the subcutaneous skin tissue of each treatment group ($P < 0.001$). Furthermore, tumors treated with both doses of hyperthermia showed a significantly reduced IFP in comparison to the IFP of control tumors ($P < 0.001$) (Fig. 2).

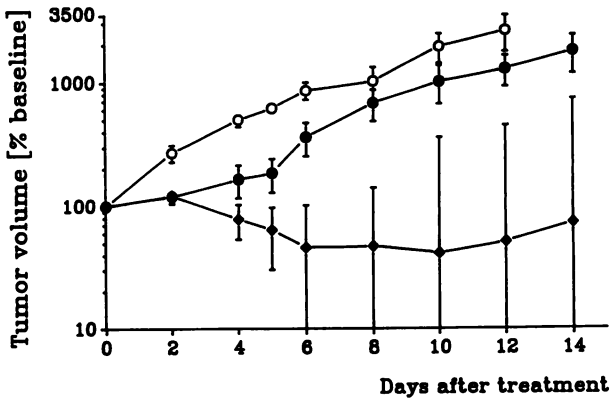

Fig. 1. Relative changes in tumor volume of A-Mel-3 tumors without treatment (○; $n = 6$), after treatment with hyperthermia at 43.0°C for 30 min (●; $n = 6$) or 60 min (◆; $n = 6$). Tumors receiving hyperthermic treatment for 60 min demonstrated partial tumor response, whereas tumors treated for 30 min only showed a growth delay of 2.7 days in comparison to exponential growth of the control tumors (median ± SE of the median).

Table 1 *Tumor growth time of the amelanotic hamster melanoma (A-Mel-3) after hyperthermic treatment at 43°C for 30 and 60 min*

Data are presented as the median ± SE of the median. Growth time of tumors treated for 30 min was significantly prolonged in comparison to the controls ($P < 0.01$). Tumors treated for 60 min never reached the defined tumor volumes within the observation time.

	Time (days)		
Volume (cm^3)	Control ($n = 6$)	Hyperthermia 43°C, 30 min ($n = 6$)	Hyperthermia 43°C, 60 min ($n = 6$)
0.25	0.60 ± 0.17	1.30 ± 1.44	>14
0.50	2.20 ± 0.14	5.60 ± 2.14	>14
1.00	4.00 ± 0.32	7.00 ± 0.89	>14
2.00	8.10 ± 0.29	10.00 ± 1.01	>14

Table 2 *Tumors showing complete response, partial response, and no response*

A complete response was not observed in any group. Only tumors treated with 43°C for 60 min showed a partial response. No change in tumor mass was seen in two of six tumors of both treatment groups, whereas all tumors in the control group increased in volume.

	Complete remission	Partial response	No response
Control	0/6	0/6	0/6
Hyperthermia 43°C, 30 min	0/6	0/6	2/6
Hyperthermia 43°C, 60 min	0/6	4/6	2/6

[4] In a limited number of experiments ($n = 3$), both IFP and tumor volume increased by a factor of 2 during the course of hyperthermia (43°C for 60 min). However, both of these parameters returned to their baseline values within 1 h after treatment. These results are consistent with the development of interstitial edema seen by other investigators (9).

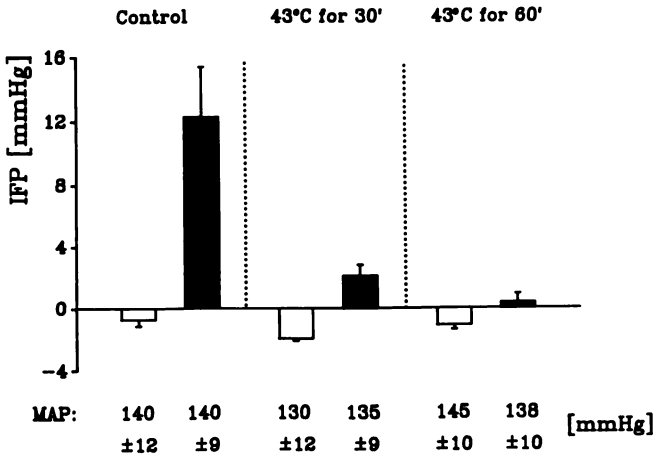

Fig. 2. Interstitial fluid pressure of tumors and untreated skin tissue of the control group, 48 h after treatment with hyperthermia at 43.0°C for 30 min and at 43.0°C for 60 min (median ± SE of the median). No significant differences of the IFP in skin (□) were found between the three groups, whereas interstitial fluid pressure in tumors (■) following local hyperthermia differed significantly when compared to controls ($n = 9$; $P < 0.001$). The IFP in tumors receiving a higher hyperthermia dose (43°C for 60 min; $n = 14$) was more decreased than in tumors receiving a lower dose (43°C for 30 min; $n = 14$; $P < 0.05$).

The mean IFP of control tumors was 12.6 mmHg (median = 12.3, SE = 3.1, $n = 9$). The IFP in tumors 48 h after local hyperthermia decreased significantly with prolonged hyperthermic treatment (43°C for 30 min *versus* 43°C for 60 min; $P < 0.05$) (Fig. 2). In tumors treated with 43.0°C for 30 min the mean IFP was 2.8 mmHg (median = 2.1, SE = 0.7, $n = 14$), and for 60 min the IFP was 0.9 mmHg (median = 0.4, SE = 0.6, $n = 14$).

Discussion

The interstitial hypertension in tumors may be a physiological barrier to the delivery of antibodies and other macromolecules used for cancer detection and treatment (10). Therefore, it is important to develop strategies to lower IFP. The present work shows that the A-Mel-3 amelanotic melanoma (100–200 mm^3) exhibits IFP in the range of other experimental and human tumors (1–6). The only exception to date is the human malignant melanoma, which has shown pressures as high as 48 mmHg for very large lesions (100 cm^3). Whether this difference in pressure is due to the size or biological variability is not known.

Several investigators have shown that localized HT at an appropriate dose can impair tumor microcirculation (11–14). This thermal dose varies from one tumor type to another. For A-Mel-3, the thermal dose for a pronounced blood flow reduction is 15 min at 42.5°C (12). Therefore, at the thermal dose used in our current study the tumor microcirculation is likely to be impaired in a dose-dependent manner, and hence local microvascular pressure in tumors is likely to come close to zero. As shown by Jain and Baxter (15, 16) theoretically and verified by Boucher *et al.* (3) experimentally, the driving force for the elevated IFP in tumors is the local microvascular pressure. Therefore, it seems reasonable to assume that the lowered IFP in tumors is a result of vascular damage in these tumors due to HT.

The reduction in IFP at 48 h after HT may also explain the increased and more uniform delivery of monoclonal antibodies in tumors due to localized HT (17–19). At the onset of heating, blood flow in tumors may increase, bringing in more MAB to the tumor. After a period of heating, when reduction or cessation of blood flow begins, antibodies are likely to remain trapped in the tumor. When the IFP is elevated, the MAB cannot extravasate adequately, due to the reduced transvasuclar convection. In addition the monoclonal antibodies extravasated in the periphery are "washed out" into the surrounding normal tissue (15, 16). Reduced IFP may increase MAB delivery by facilitating extravasation and by reducing the washing out of the trapped MAB into the surrounding tissue. Furthermore, any increase in the diffusive component of vascular permeability due to hyperthermia would also enhance extravasation (20, 21). Further experiments are now needed to verify our hypothesis regarding the role of IFP changes during hyperthermia in MAB delivery.

An important and useful finding of the current investigation is that the IFP of tumors treated with higher thermal doses (43°C for 60 min) was significantly lower than that of tumors treated with a lower dose (43°C for 30 min), even though there was no significant difference in tumor size in these two treatment groups (Figs. 1 and 2). Thus IFP may reflect the degree of damage to tumor microcirculation, long before this damage shows up as changes in tumor volume (Fig. 2). This novel result along with those of Roh *et al.* (4) suggest that changes in IFP may be useful in predicting treatment outcome and in individualizing strategies for treatment. Further investigations are now needed to generalize these findings.

Acknowledgments

The skillful assistance of Beatrice Sonntag and Christine Csapo and the helpful comments of Yves Boucher and Laurence T. Baxter on the manuscript are gratefully acknowledged.

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