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Influence of differences in tumor vascularity upon the effects of hyperthermia

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Summary. Utilizing two types of human renal carcinoma heterotransplanted in nude mice, we investigated the variations in hyperthermic effects (42.5 °C for 30 min) caused by differences in tumor type with special reference to variations in tumor vascularity. In the hypovascular JRC1 strain, sporadic vascular dilation was observed throughout the tumors after heating. Destruction of tumor cells was observed mainly in the region of dilation. In the hypervascular JRC11 strain, homogenous vascular dilation was observed immediately after heating, mainly at the periphery of tumors. There was a decrease in the viability of cells in the center of the tumor. Therefore, the hypervascular tumors showed greater destruction mainly at the center where blood circulation was reduced. The range of necrosis was also greatly affected by the extent of vascular dilation caused by heating in hypovascular tumors.

Key words: Renal carcinoma – Vasculature – Histological change – Hyperthermia – Nude mice

Hyperthermia is employed in the treatment of cancer and has been found to have unique anti-tumor effects not seen with previous treatment methods [3, 12]. However, the clinical application of hyperthermia remains difficult, depending on the organ being treated. There are two reasons for this. One is that the development of effective heating devices, including those for temperature measurement, is still not advanced enough. The second is that the character of the response against different types of tumor cannot be assessed in cases of concomitant use because the differences in hyperthermic effects due to differences in tumor type are not yet sufficiently understood.

Because of its importance in clinical application we investigated the differences in hyperthermic effects due to differences in tumor type, with special reference to the vascularity in renal carcinoma.

Materials and methods

Experimental animals and tumor strains used

The mice used in this study were BALB/C nu+/nu+, male, 6–8 weeks of age (Klea Japan). They were reared in a specific pathogen free (SPF) environment in the Laboratory Animal Center of the author's university. The tumor strains used in this study were two types of human renal carcinoma (JRC1, JRC11) serially heterotransplanted in nude mice of completely different types established and maintained in the authors' laboratory. The JRC1 strain showed a papillary pattern in structural organization. It was hypovascular in comparison with JRC11 strain. This grade III tumor strain (medium degree of differentiation) was comparatively slow growing, with a doubling time of 9.87 days (Fig. 1A). The JRC11 strain showed a mixture of anaplastic and alveolar structural organization and was hypervascular. It was a rapid growing, grade IV renal carcinoma strain (low degree of differentiation or undifferentiated) with a doubling time of 2.74 days (Fig. 1B).

Temperature measurement and heating methods

Temperature was measured with a thermosensor using the thermodependent transmission of light through a gallium-arsenic crystal. The hyperthermic device was manufactured by Clini-Therm Co. The core of the tumor was subjected to 915 MHz microwave hyperthermic treatment by means of an antenna inserted via a 16-gauge puncturing catheter, which was connected to a Mark VII generator (Fig. 2A).

Establishment of the thermal dose

When the weight of the implanted tumor had reached 1,500–1,700 mg, the experiment was started under nembutal anesthesia. The temperature was determined by regulating the microwave power applied to the center of the penetrating catheter, whereby the lowest temperature at the periphery of the tumors in all experiments was 42.5 °C (Fig. 2B, C). A similar temperature distribution was obtained in the two tumors when the tumor size was the same.

Changes after hyperthermia

For both the JRC1 tumors, nude mice were killed immediately, or 1, 6, 12, 24, 48 and 72 h and 7 days after heating, and the

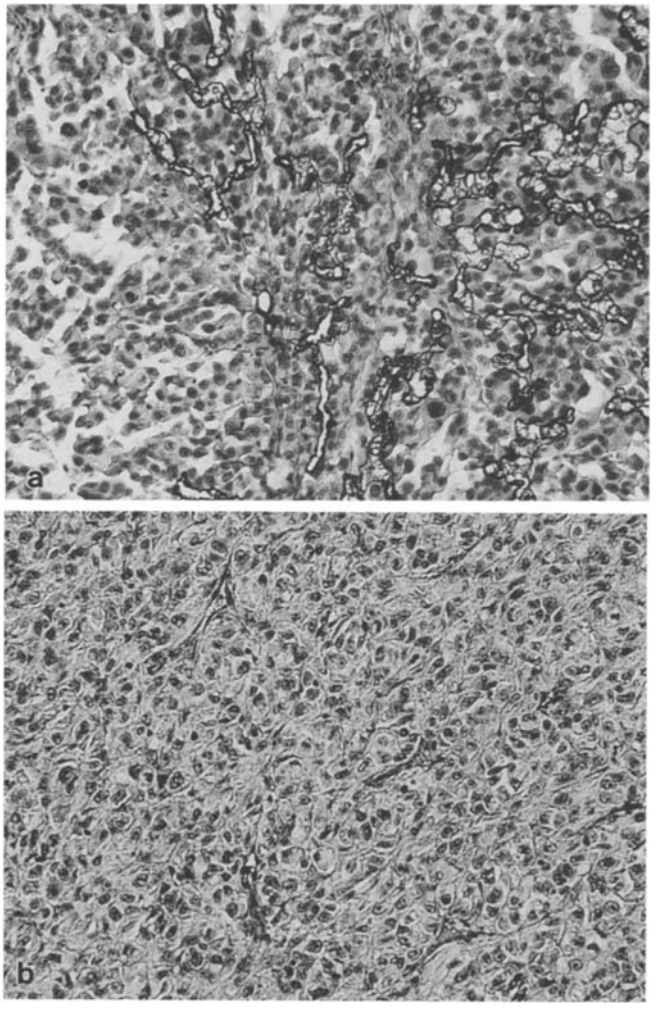


Fig. 1. **a** The histological type of this heterotransplantable human renal carcinoma shows a papillary pattern with grade III malignancy (JRC1; H&E staining, ×100). **b** The histological type of this heterotransplantable human renal carcinoma shows an anaplastic, partly alveolar pattern with grade IV malignancy (JRC11; H&E staining, ×100)

excised tumors were observed by light microscopy after H&E staining.

In each experiment, five mice were made up one group. If two or more mice died in one group, the experiment was excluded from the study, and the same experiment was performed.

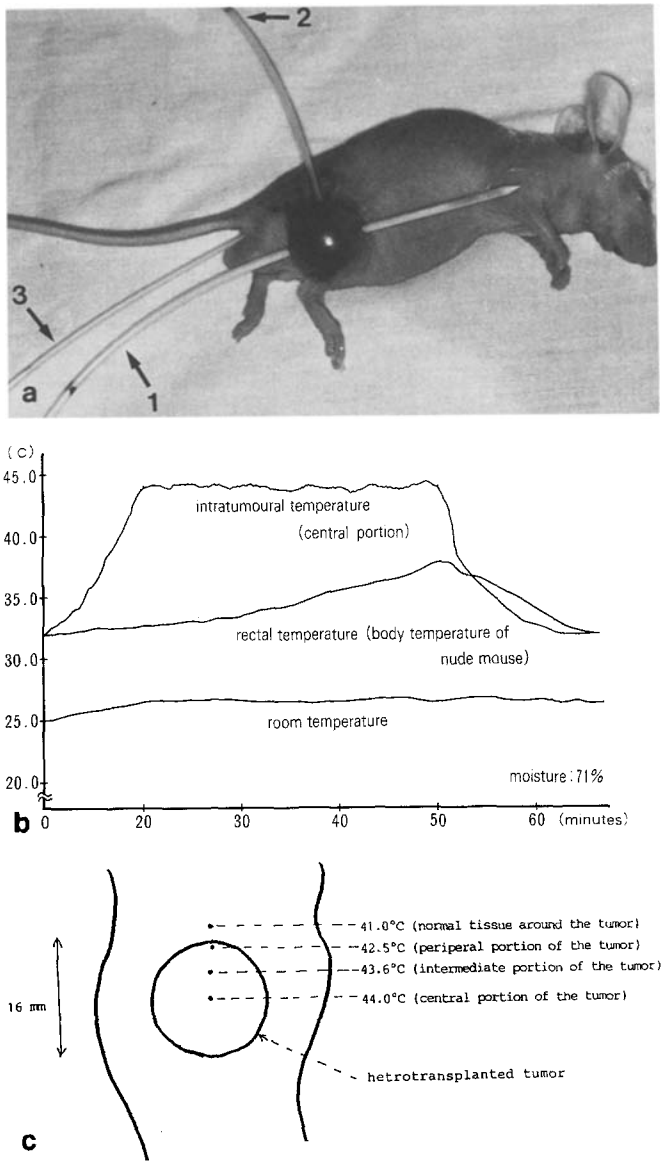


Fig. 2. **a** Method of hyperthermia. *1* An antenna inserted into the center of the tumor and connected to a Mark VII generator; *2* thermosensor for tumor; *3* thermosensor for body temperature of nude mouse. **b** Graph shows monitoring of the tumor temperature and body temperature of the mouse during the hyperthermic experiment. **c** Temperature distribution in and around the tumor during heating

Results

Sequential changes in the JRC1 strain

Histology immediately after heating. There was mild, sporadic vascular dilation from the periphery to the center of the tumors. There was no difference in the degree of vascular dilation between the center and periphery of the tumors, and there were no changes in the tumor cells (Fi. 3A). In detailed observations, both dilated and non-dilated tumor vessels were present.

Histology 1 h after heating. The changes were similar to those seen immediately after heating.

Histology 6 h after heating. There wer no major differences compared to the histology seen immediately after heating.

Histology 12 h after heating. Compared with the histology 6 h after heating, there was a reduction in the viability of the tumor cells (a tendency toward swelling of the nuclei and cytoplasm) in the center of the regions showing vascular dilation and a necrotic tendency (the ischemic changes characterized by coagulation necrosis), but the viability of the tumor was maintained around the thick stroma with no vascular dilation.

Histology 24 h after heating. Compared with the histology

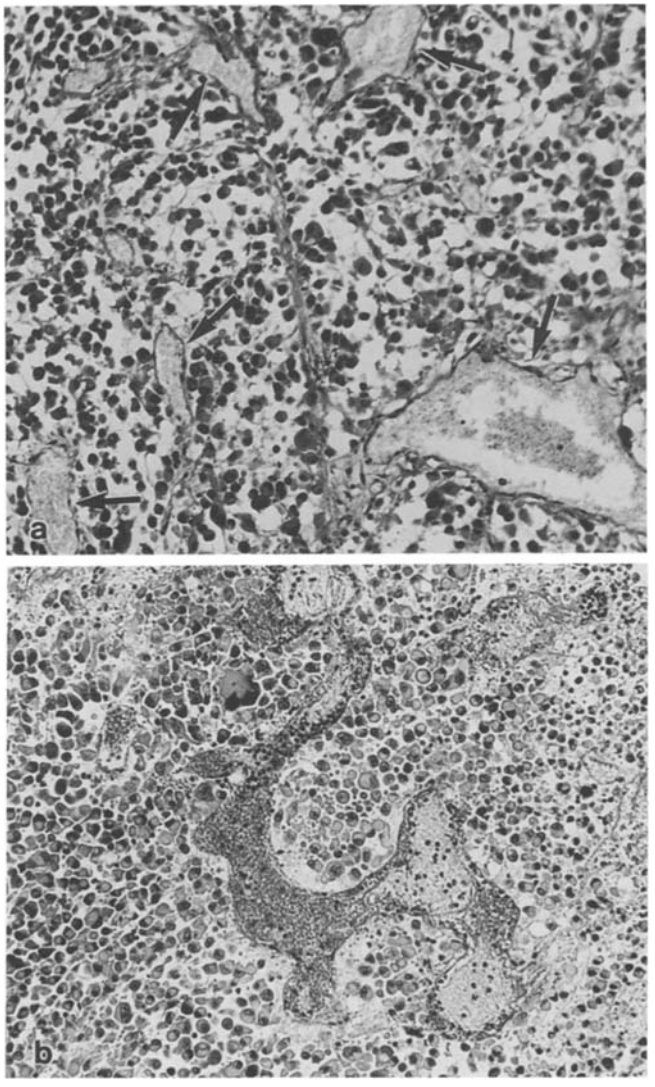


Fig. 3. a Light microscope appearance of JRC1 strain just after heating. Sporadic vascular dilatation (*arrow*) and blood stasis are seen in the tumor tissue (H&E staining, ×100). **b** Forty-eight hours after heating of JRC1 strain. Tumor cells around the dilated blood vessels show coagulation necrosis, and there are islands of viable tumor cells surrounded by these degenerative areas. (H&E staining, ×100)

12 h after heating, there was pyknosis of the nuclei of the tumor cells around the sites of the vascular dilation and destruction of the tumor structure.

Histology 48 h after heating. The destruction in the areas of vascular dilation had progressed and coagulation necrosis had developed. The viable tumors had formed single masses and were sporadically seen in the form of islands (Fig. 3B).

Histology 72 h after heating. About two-thirds of the tumor cells showed degenerative necrosis, but the tumor cells that retained their viability were grouping.

Histology 7 days after heating. The tumor cells, mainly at the periphery of the tumors, showed coagulation necrosis, but this area had decreased to about one-third of that present 72 h after heating, while the viable tumor cells expanded in the direction of the necrotic foci.

These results indicated that the JRC1 strain there was tumor vascular dilation sporadically throughout the tumor immediately after heating, but there were also non-dilated tumor vessels, mainly in the thick stroma. Beginning 24 h after heating the tumor cells started to show pyknosis of the nuclei and disappearance of the cytoplasm mainly in the regions of tumor vascular dilation. However, 72 h after heating, the viable tumor cells again started to grow, mainly in the areas without vascular dilation, and almost two-thirds had recovered 1 week after heating.

Sequential changes in the JRC11 strain

Histology immediately after heating. Marked vascular dilation was observed in the periphery of the tumors. In the centers, the vascular dilation was not as severe as at the periphery. There were no changes in the tumor cells (Fig. 4A).

Histology 1 h after heating. At the periphery of the tumors, the vascular dilation was more marked than that immediately after heating, but the vessels in the center had no fresh blood even in the dilated regions (Fig. 4B).

Histology 6 h after heating. At the periphery of the tumors, there was vascular dilation and extravasation associated with partial destruction of the blood vessels, but there were no apparent changes in the tumor cells. However, there was pyknosis of the nuclei and vacuolation of cytoplasm in the center of the tumors.

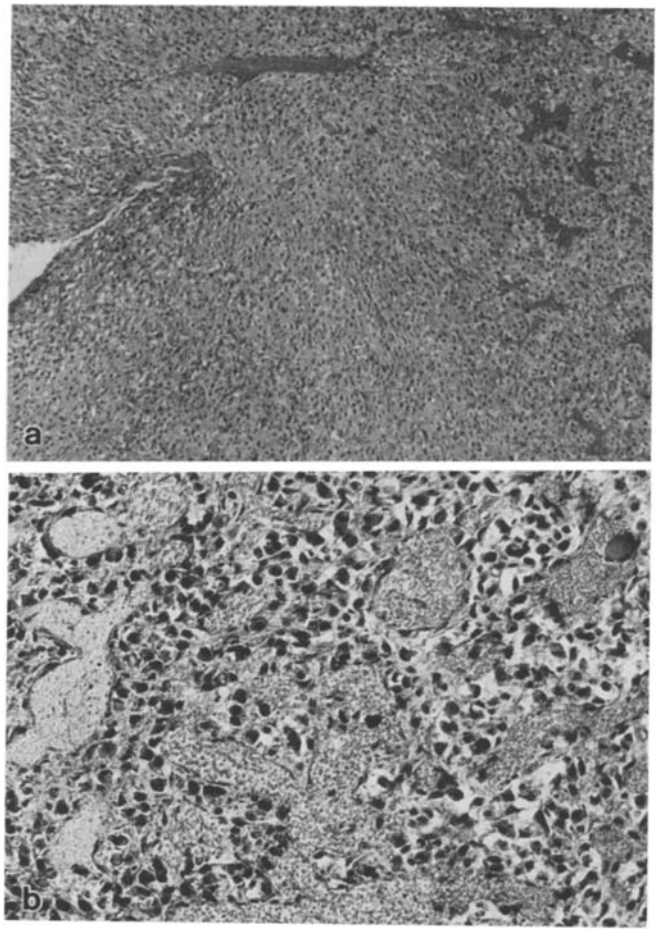
Histology 12 h after heating. There were no changes in the periphery of the tumors, but there was a decrease in cell stainability and partial necrosis nearer to the center of the tumors. The cells in the center of the tumors had lost their viability and partial necrosis had occurred.

Histology 24 h after heating. The histology was almost the same as that 12 h after heating, but necrosis of cells in the center of the tumor had progressed.

Histology 48 h after heating. The histology was basically the same as that after 12 h, but there was blood flow in the dilated vessels at the periphery of the tumors.

Histology 72 h after heating. Degeneration of the center of the tumor had progressed, but the vascular dilation at the periphery of the tumors had become slight, the tumor cell viability was maintained, and there was considerable tumor growth, mainly in this area.

Histology 7 days after heating. The center of the tumor was necrotic and there was growth mainly at the periphery of the tumors, with viable tumor cells surrounding necrotic



foci. Vascular dilation at the periphery was very slight and the tumor histology resembled that of the control group.

These results indicated that there was marked vascular dilation at the periphery of the tumors from immediatly after heating, but the blood flow to the center of the tumors was reduced. These trends progressed and by 12 h after heating, there was degeneration of the tumor cells in the center, which developed into necrosis. However, 72 h after heating, the vascular dilation at the periphery had almost disappeared and the tumor was growing in this area. One week after heating, tumor growth continued in a donut-shape surrounding the necrotic center. Table 1 gives a summary of the sequential changes in the two strains after heating. In this study, there were no cases in which the tumor cells completely disappeared due to hyperthermia alone.

Discussion

Hyperthermia has recently been attracting considerable attention as a fourth method of cancer therapy after surgery, chemotherapy and radiation therapy. The anti-

Fig. 4. a Histological changes just after heating of the JRC11 strain. Tumor vessels show uniform dilation at the periphery of the tumor (the peripheral portion of the tumor is in the upper right part of this picture; H&E staining, ×40). **b** Histological changes of JRC11 tumor 1 h after heating. Blood flow in the dilated vessels at periphery of the tumor can be seen (right half of field), but no fresh blood flow in the dilated vessels in the deep portion of the tumor (left half of the field; H&E staining, ×200)

Table 1. Sequential histologic changes in the JRC1 and JRC11

Time after heating	JRC1	JRC11
Immediately after	Mild, sporadic vascular dilation from the periphery to the center	Marked vascular dilation in the periphery of the tumors
1 h	No major differences from the histology immediately after heating	At the periphery of the tumor, the vascular dilation was more marked than immediately after heating
6 h	Similar to those seen 1 h after heating	Extravasation at the periphery, pyknosis of the nuclei and vacuolation of cytoplasm in the centre of the tumor
12 h	Reduction in the viability of the tumor cells around the dilated vessels, but not around the thick stroma without vascular dilation	The cells in the center of the tumors lost their viability
24 h	Destruction of the tumor cells around the sites of vascular dilation	Necrosis of the cells in the center of the tumor had progressed
48 h	The viable tumor cells had formed single masses and were seen in the form of islands	Same as 24 h after heating
72 h	The tumor cells that retained their viability were grouping	Considerable tumor growth was observed around necrotic areas
7 days	The viable tumor cells expanded in the direction of the necrotic foci	Tumor growth in a donut-shape surrounding the necrotic center of the tumor

tumor effects of hyperthermia have been reported on the basis of in vitro and in vivo fundamental investigations using many tumors and animals [7, 9]. From studies of data including clinical studies, it has been found that the fundamental action of hyperthermia in vivo is based on the fact that tumor tissue shows a higher sensitivity to heat than normal tissue [3]. These differences in sensitivity are assumed to be closely related to differences in the changes in normal and tumor vascularity during heating, namely, differences in blood flow, pH, oxygen pressure, vascular volume and other physiological changes [4, 8, 10].

The two strains of human renal carcinoma serially transplanted in nude mice used in the present study have different properties, especially in neovascularity. The JRC1 strain is a hypovascular tumor because tumor morphology showed a papillary pattern [6], while the JRC11 strain is a hypervascular tumor characteristic of renal carcinoma, that is to say, it is one that has more abundant vasculature than normal tissue. The authors studied histopathological changes in these two strains after heating under the same hyperthermic environment. Attempts were made to clarify differences in hyperthermic effects between these two strains.

With respect to morphological and functional changes in the vascularity due to hyperthermia, it appears from various studies that the occurrence of some sort of disorder in the vascularity results in wide ranging changes within the tumor [4, 5]. Eddy [2] observed hyperthermic changes microscopically using cheek-pouch chambers (squamous cell carcinoma) transplanted in hamsters and found that there was vascular dilation 15 min after heating at 43 °C for 30 min and that dilation of vasculature continued to be severe, especially at the periphery of the tumors, even after the temperature had returned to 34 °C. In the present study, marked vascular dilation and stasis were observed from just after heating of the hypervascular JRC11 strain, which agreed with the results of Eddy [2]. From the pathophysiological viewpoint, the effects of the tumor vasculature are an important in vivo response to hyperthermia.

According to the work of Rappaport [10] on the analysis of changes in intratumoral blood flow after heating, the blood flow showed a marked decrease after heating at 43.5 °C for 60 min, and it had not recovered after 16 h. Therefore, from the reports of Eddy [2] and Rappaport [10], and also from the results of the present study, it is evident that tumor vascularity differs from that of normal tissue and that dilation of the vasculature occurs after heating. As a result, especially in hypervascular tumors, the vascular floor was dilated mainly at the periphery of the tumor where stasis of blood flow resulted. It became evident that effective circulation was lacking mainly at the center of the tumors.

With respect to changes in the JRC11 tumor cells, pyknosis of the nuclei and disappearance of the cytoplasm, mainly in the tumor centers, became noticeable 6 h after heating. These changes can be interpreted as secondary hypoxic changes of the cells in the center of the tumor due to the vascular destruction associated with the decreased blood flow. This was also clear from the reduced stainability of erythrocytes in the dilated blood vessels in the intermediate region between the periphery and the center [11].

Hypovascular cancers with a papillary pattern are characterized histologically by growth with cancer cells attached like bunches of grapes to one or several fibrovascular stromas [1]. The appearance of vascular dilation due to hyperthermia immediately after heating was the same as the JRC11 strain, but this dilation was not uniform in the JRC1 strain. When considered in more detail, the blood vessels in the stroma showed almost no dilation immediately after heating and even later, but there was marked dilation of the fibrovascular channels branching off from the stroma. Thus, it appeared that this dilation was connected with the macular necrosis observed at 72 h mainly in the regions of sporadic vascular dilation.

The main question here is why was the uniform tumor vascular dilation seen in the JRC11 strain in the periphery not observed in the JRC1 strain.

Tumor vasculature differs from that of normal tissue. Structurally, there is only one thin layer of endothelial cells and there are no receptors or nerve control because of the lack of muscle layers or connective tissue in the vessel walls. Therefore, there is only passive reaction to strong external forces such as hyperthermia [12]. However, the papillary pattern consists of a stroma in which the tumor blood vessels are surrounded by fibrous connective tissue. Compared with the exposed condition of the fibrovascular channels in other tumor vessels, it appears that this strain is not as susceptible to changes such as vascular dilation with heating, especially in the area of thick stromas. This is assumed to cause the selectivity seen in the tumor vascular damage due to heating.

The vascularity of tumors appears to undergo changes that differ according to the degree of heating, the duration and range of heating, and the heat sensitivities of respective tumor cells. In this study, it was evident that there are major changes due to hyperthermia related to differences in the tumor vascularity. The range of the degeneration of tumor cells associated with heating is greatly affected by microvascular dilatation due to the heating. It is, however, very difficult to obtain homogeneous temperature distribution utilizing our 915 MHz microwave device because this may cause a hot spot in the center of the transplanted tumor. Further study to investigate the effects of a hyperthermic method that can provide homogeneous temperature distribution on the tumor morphology of various types of tumor is underway.

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