Histological Analysis of the Effect of Hyperthermia on Normal Rabbit Hepatic Vasculature

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ABSTRACT

The effects of hyperthermia on rabbit hepatic vasculature were studied histologically. To investigate heat-induced vascular damage in the central veins, portal veins, and hepatic arterioles, the left lobes of rabbit liver were heated locally for 30 min in the range of 40–46°C. Hyperthermia was induced by an 8-MHz radiofrequency current heating device using a needle type interstitial applicator. This device allowed application of heat to a central area of 10 x 10 mm no more than 1°C below the preset temperature. Within the area of 1 cm², the percentage of damaged (ruptured or thrombosed) vessels was estimated for each type of hepatic vasculature. Vascular damage following hyperthermia continued up to 24 h after heating for the three types of hepatic vasculature. Central veins were the most thermosensitive followed by portal veins, whereas hepatic arterioles were the most thermoresistant. The temperature causing 50% vascular damage 24 h after heating was 41.5–42.5°C, 42.5–43.5°C, and 44–45°C for central veins, portal veins, and arterioles, respectively. This differential thermal responsiveness of hepatic vasculature may be attributed to the histological structure of the vessels.

INTRODUCTION

Heating at temperatures slightly above the body temperature evokes a significant increase in blood flow and vasodilation in normal tissues, and at higher temperatures vascular stasis and collapse occurs (1–3). However, our knowledge of the vascular changes in normal tissue is limited to those in the skin and muscle, primarily owing to the lack of a proper device to heat other organs.

We have developed interstitial radiofrequency heating applicators that can heat deep-seated organs relatively homogeneously (4). The liver seems to be a good model for studying the vascular changes following hyperthermia because it contains 3 different types of vessels. In the present study, thermal injury to the central veins, portal veins, and arterioles of the rabbit liver was investigated histologically.

MATERIALS AND METHODS

Animals. Twenty-eight healthy Japanese white rabbits weighing approximately 3 kg were used. They were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were kept in the Animal Center of Kyoto University.

Heating Procedure. The animals were anesthetized with pentobarbital sodium at a dose of 20–25 mg/kg by i.v. injection. Under anesthesia, the left lower lobe of the liver was exposed by operation, and a needle type interstitial RF¹ heating applicator was inserted into the liver (Figs. 1 and 2). Hyperthermia was induced by an 8-MHz RF current heating device with maximum RF output of 200 W (Yamamoto Vinitor Co., Ltd., Osaka, Japan). The heating device consisted of an 8-MHz RF generator unit, a thermometry unit, an interstitial applicator, and an automatic on-off switching control system. Impedance matching of RF current could be controlled manually.

Temperature Distribution. The temperature distribution of the liver heated by the device was described previously (4) and will be described here briefly. Temperatures were measured with thermocouple sensors through 21-gauge angiocatheters that were inserted into the liver along a X-axis, and the temperature distribution was obtained by moving thermocouples. Generally, the temperature at the center of applicator was equilibrated within 2–3 min after the start of heating, and a steady state temperature was maintained by an automatic on-off switching system thereafter. During the steady state, temperatures 4 mm apart the center along the Y-axis were 0.5–0.7°C below the preset central temperature, and temperatures 5 mm apart the center along the X-axis were 0.7°C below the central temperature. Thus, a central area of 10 x 10 mm could be heated no more than 1°C below the preset temperature with this applicator. Liver injury caused by the needles was negligible.

Treatment Schedule. The liver was heated at 40, 42, 44, and 46°C for 30 min. The animals were killed with pentobarbital sodium immediately, 3 h, and 24 h after the treatment. For the control group, the animals were killed immediately and 24 h after a sham treatment without hyperthermia. Each experimental group consisted of 2 rabbits.

Evaluation of Vascular Damage. Two 3-mm-thick slices were sectioned from the treated liver (Fig. 1). The liver slices were fixed with 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin.

For evaluation of vascular damage, the center of the heated area (10 x 10 mm) was photographed at x25. This panoramic picture was used as a sort of map of each section. Within the 10–x 10-mm area, the number of ruptured or thrombosed vessels (D) and the total number of vessels (V) were counted for each type of hepatic vasculature under a microscope at x20 or x50. Dilated or congested vessels were not regarded as damaged vessels. Then, the percentage of vascular damage was calculated as

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\frac{D}{V} \times 100
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in each section.

RESULTS

Table 1 shows the total number of vessels in the examined area. No difference in the total number of vessels was noted according to the treatment temperature.

Fig. 3 shows the changes in percentage of vascular damage for the 3 types of hepatic vasculature. Vascular damage after heating progressed up to 24 h after the treatment in all 3 types of vessels. Immediately after heating, histological sections showed mild rupture of central and portal veins at higher than 44°C, although no vascular changes in hepatic arterioles were observed at such high temperatures. Three h after heating, marked congestion of RBC at hepatic sinusoids and rupture of portal and central veins were noted at higher than 44°C. Twenty-four h after heating, the percentage of damaged vessels was increased for all 3 types of vessels. Focal degeneration of hepatocytes was noted in sections obtained 24 h after heating at 42 and 44°C. Most of the central veins were thrombosed or destructed at 44°C, whereas undamaged portal veins and hepatic arterioles were often noted in the interlobular connective tissue (Fig. 4). Massive necrosis of hepatocytes occurred at 46°C.

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Received 1/5/89; revised 4/10/89; accepted 4/27/89.

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³Supported in part by a Grant-in-Aid for Scientific Research (61010041, 61015038) from the Ministry of Education, Science and Culture, Japan.

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¹ The abbreviation used is: RF, radiofrequency.

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were most resistant to heat (Fig. 3). The temperatures causing 50% vascular damage 24 h after heating were 41.5-42.5°C, 42.5-43.5°C, and 44-45°C for central veins, portal veins, and hepatic arterioles, respectively.

Vascular vulnerability to heat was greatest in the central veins, followed by the portal veins, and the hepatic arterioles. Nakajima et al. (10) measured the hepatic blood flow changes after regional heating in rats and reported that hepatic arterial blood flow increased by heating at 41°C and 43°C whereas portal blood flow decreased at the same temperatures. Thus, the changes of hepatic vasculature by hyperthermia differed among the types of vasculatures.

One possible explanation for the differential thermal responsiveness of the hepatic vasculature is the difference in temperature of the blood inside the vessels. The normal vasculature is generally considered to be more resistant to heat than the tumor vasculature (3). However, we found that central veins and portal veins were more thermosensitive than the reported normal vasculatures in the skin and muscle. Falk (6) reported a marked reduction in mouse jejunal vasculature after heating at 41–42°C for 1 h. Thus, the normal vasculature except for those in the skin and muscle showed thermosensitivity quite similar to those of the transplantable rodent tumors (2, 3, 8, 9).

Hepatic arterioles were the most thermoresistant among the 3 types of hepatic vasculature. Hepatic arterioles have several histological layers including endothelium, internal elastic lamina, smooth muscle cells, and adventitial loose connective tissue, whereas the wall of a central vein consists of endothelium and surrounding sparse smooth muscles. Portal veins pass through the interlobular connective tissue. Previously, we found that the tumor vasculature with perivascular connective tissues is more thermoresistant than that composed only of sparse endothelial cells (7, 8). Thus, the differential thermosensitivity of vessels may cause cooling of portal veins and arterioles. However, the marked difference in thermal responsiveness between portal veins and arterioles could not be explained by the possible difference in temperature in the blood inside the vessels.

Histologically, the 3 types of hepatic vasculature differ significantly. For example, hepatic arterioles have several histological layers including endothelium, internal elastic lamina, smooth muscle cells, and adventitial loose connective tissue, whereas the wall of a central vein consists of endothelium and surrounding sparse smooth muscles. Portal veins pass through the interlobular connective tissue. Previously, we found that the tumor vasculature with perivascular connective tissues is more thermoresistant than that composed only of sparse endothelial cells (7, 8). Thus, the differential thermosensitivity of vessels may be attributed to the histological structure of vessels. We consider that the histological structure of vasculatures is quite important in determining the thermosensitivity of tumor vessels as well as normal vessels.

Histopathological autopsy observations of patients who had died of hyperpyrexia revealed centrilobular necrosis of hepato-
Fig. 3. Changes in percentage of vascular damage following hyperthermia. Each point represents the mean ± SD (bars) of 4 sections.

Fig. 4. A histological section of rabbit liver obtained 24 h after heating at 44°C for 30 min. The endothelial lining of central vein was broken (open arrow), and thrombotic materials were noted in it, whereas a patent and undamaged portal vein (arrowheads) and hepatic arteriole (arrow) were observed in the interlobar connective tissue. H & E, x33.

cytes 16–60 h after hyperthermia, with relatively undamaged hepatocytes at the periphery of the lobules that underwent active regeneration (11–13). It is very likely that this high thermosensitivity of hepatocytes in the centrilobular region is a secondary result of heat-induced vascular damage to central veins, which were the most thermosensitive hepatic vasculature. This finding supports the hypothesis that reduction in blood flow by hyperthermia may be responsible for tissue necrosis.

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