EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Morphological Changes in the Liver after Microwave Destruction

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 131, No. 4, pp. 448-452, April, 2001
Original article submitted September 20, 2000

We studied morphological changes in the liver after local MW-destruction. Mircowave radiation damaged vascular walls with the formation of extensive necrotic focus; demarcation zone appeared 24 h after exposure, encapsulation occurred after 7 days, and replacement with a fine cicatrix formed by mature connective tissue after 4 months.

Key Words: *MW-destruction; liver; experiment*

Use of new technological equipment is a perspective trend in pediatric surgery. An original method for treating benign vascular tumors (hemangiomas) of intricate anatomical localization has been developed at the Pediatric Surgery Hospital of Russian State Medical University. The method is based on the use of MW radiation (embolization+MW destruction protocol [4]) and is an alternative to complex traumatic surgical interventions. Sixty-two children aged 3-12 months with extensive angiomas of the parotid area were operated using this method. Positive results were attained in all cases: the tumor completely regressed within 5-6 months and was replaced with fine cicatrix. Microwave therapy was effectively used in patients with angiomas [2].

However, biological effects of MW radiation in a destruction mode (5 W/cm^2 power density, 45-60°C tissue temperature) are little studied.

Microwave-induced hyperthermia (1.2-1.3 W/cm^2 power density, 40-44°C tissue temperature), the most vigerous regimen of MW radiation used in medicine, was applied for the treatment of malignant tumors [1,3,7-12]. The mechanism of damaging effect of microwaves remains unclear: hypotheses of specific thermal effect, nonthermal effect, nonthermal protein coagulation, impairment of tissue function regulation, *etc*. are discussed [3,7-9,12]. On the other hand, it is known that hyperthermia induces selective destruction (coagulation necrosis) of tumor cells without affecting the stroma and blood vessels [10,11].

We investigated morphological changes in tissues subjected to MW radiation and peculiarities of cicatrization after this exposure.

MATERIALS AND METHODS

Experiments were performed on the liver of male Wistar rats (120-125 g), because this organ is considered to be a model of hemangioma due to similarity of thermophysical characteristics [6]. Laparotomy was carried out under ketamine anesthesia; left liver lobe was subjected to MW destruction with a Yakhta device for local hyperthermia (33 cm wavelength, 915 Hz frequency, 25 mm radiator diameter, 5 W/cm^2 power density). The temperature of exposed tissue reached 60°C, duration of exposure 1 min. The temperature

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0007-4888/04/1314-0377$25.00

was controlled with a standard temperature transducer. The animals were sacrificed after 1, 5, and 24 h, 7, 14, 30, 60, 90, and 120 days. The liver from intact rats served as the control. For histological studies the liver was fixed in 4% neutral formaldehyde (pH 7.7), embedded in paraffin, and stained with hematoxylin and eosin, according to Van Gieson, and with orsein. Morphometric studies were carried out under MOV 1-15^{X} ocular, the number of cells and vessels per standard visual field (VF) was counted at ×600.

RESULTS

Destruction of liver sinusoids, portal and central veins, arteries, *i. e.* vessels of 5-400 μ in diameter, was observed 1-5 h after MW treatment (Fig. 1). Pronounced circulatory disorders (hemorrhages, stasis, thrombosis) indicated impaired blood rheology. Hyaline-droplet and vacuolar degeneration of hepatocytes was seen. Hepatocyte cords were disorganized only at the site of immediate contact with the source of radiation. Destruction of vascular walls and hemorrhages were more pronounced at the periphery of necrotic focus than at the site of exposure.

After 24 h the necrotic area (20×6 mm) was clearly demarcated from more or less intact liver tissue and from the abdominal cavity. Two zones were clearly seen in the necrotic area: detritus surrounded by the demarcation zone (Fig. 2, *a*). Hepatocytes in the demarcation zone underwent necrosis, intercellular spaces were abundantly infiltrated with segmented neutrophils. In the detritus zone, necrosis of vascular walls (not involving fibrillar structures), thrombosis, necrosis and protein degeneration of hepatocytes, and focal leukocytic infiltration were seen.

After 7 days, the necrotic zone was encapsulated and three zones were clearly distinguished: capsule, demarcation zone, and detritus zone (Fig. 2, *b*). The capsule (2.0-2.5 mm thick) consisted of fine granulation tissue of different degree of maturity, enriched with capillaries (5-10 per VF), young fibroblasts, and infiltrated with macrophages and neutrophils. It is noteworthy that the capsule contained giant multinuclear cells and foreign body granulomas starting from day 7. Demarcation zone adjacent to the capsule contained necrotic hepatocytes and was abundantly infiltrated with segmented neutrophils. Detritus zone shrank to 15×4 mm. Groups of segmented neutrophils were sometimes seen at the periphery of detritus, while its bulk was not infiltrated.

This zonal structure of the necrotic focus persisted for 60 days after MW treatment. The size of necrotic focus markedly decreased: on day 14 it shrunk to 9×8 mm, on day 30 to 5×3 mm, and on day 60 to 3.5×2.5 mm. The capsule became thinner and more mature: on day 14 it was 1.0-2.5 mm thick, on days 30 and 60 0.3-0.5 mm thick. This was paralleled by shrinkage of the detritus zone: 5×3 mm on day 14, 4.5×2.0 mm on day 30, and 3×2 mm on day 60. The width of the demarcation zone remained unchanged (0.45-0.48 μ). This zone disappeared by day 90 of the experiment.

After 90 days necrotic focus shrunk to 0.8×1.0 mm and consisted of the capsule and detritus zone. The capsule (0.16-0.40 mm thick) consisted of fine fibrous connective tissue, was well vascularized (3-5 capillaries per VF) and infiltrated with macrophages, siderophages, and giant cells (Fig. 3). The detritus zone was characterized by abundant vascularization (3-7 capillaries per VF) and macrophage infiltration (15-35 per VF), giant cells were also seen in this zone (1-6 per VF). Only solitary detritus islets were seen in the infiltrate. Foreign body granulomas with giant multinuclear cells were seen. Solitary segmented neutrophils were located mainly perivascularly. At this stage detritus was resorted mainly by macrophagal cells.

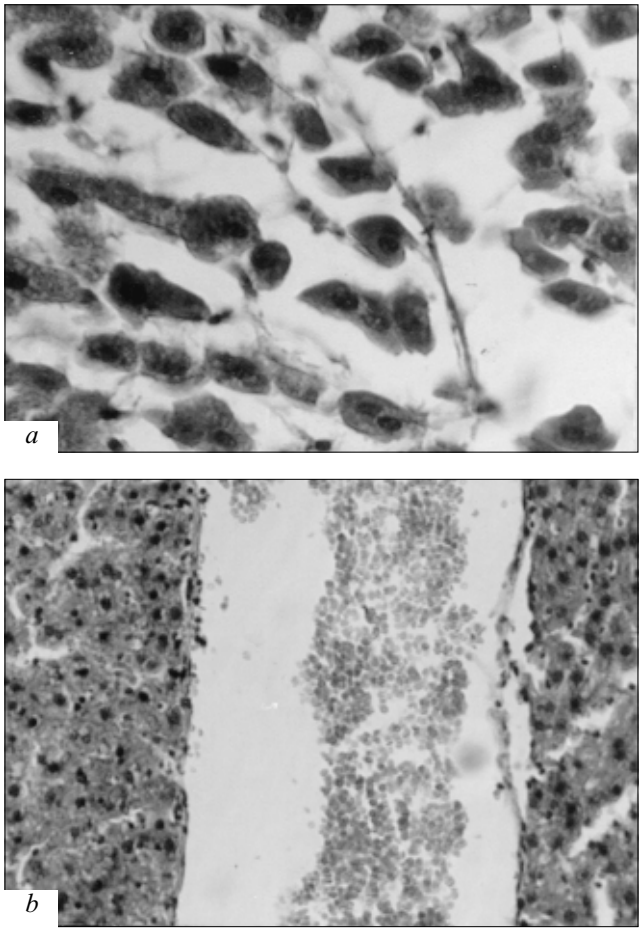

Fig. 1. Morphological changes in the liver 1 h after MW destruction. *a*) destruction of sinusoidal walls, fragmentation of hepatic cords. Van Gieson staining, ×200. *b*) destruction of portal vein walls, hemorrhages. Hematoxylin and eosin staining, ×80.

After 120 days only a fine cicatrix (0.16-0.40 μ) consisting of mature connective tissue was seen at the site of exposure (Fig. 3). No detritus was seen. Collagen fibers were fine (5-8 μ), twisted, with elastic fibers between them. The cicatrix contained numerous fibroblasts (30-35 per VF), was well vascularized, but the

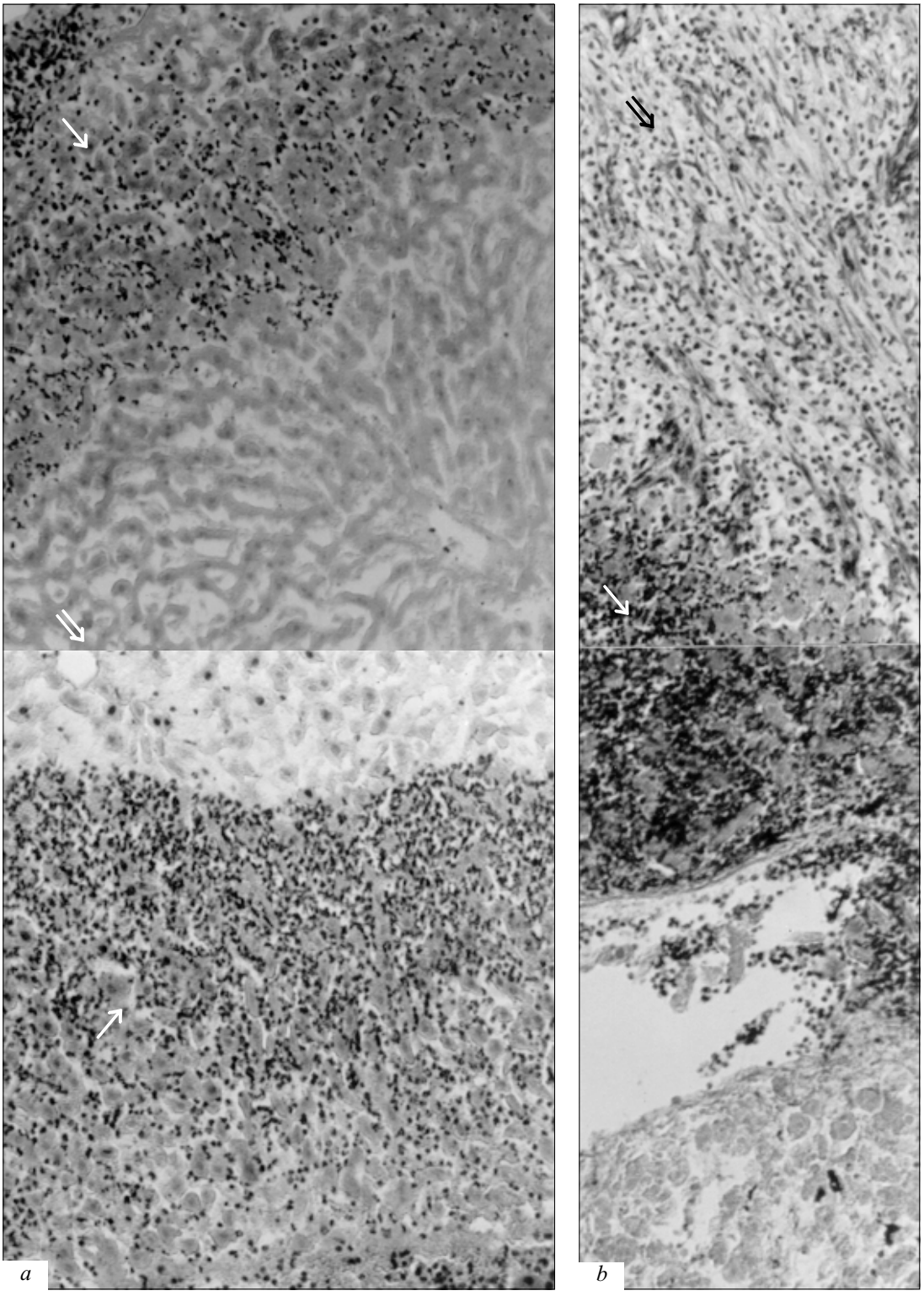

Fig. 2. Structure of necrotic focus formed after MW destruction in the liver. Hematoxylin and eosin staining, ×80 (*a, b*). *a*) 24 after exposure. Demarcation zone in the necrotic focus (arrow), surrounding detritus zone (double arrow); *b*) 7 days after exposure. Capsule, demarcation zone (arrow), and detritus zone (double arrow).

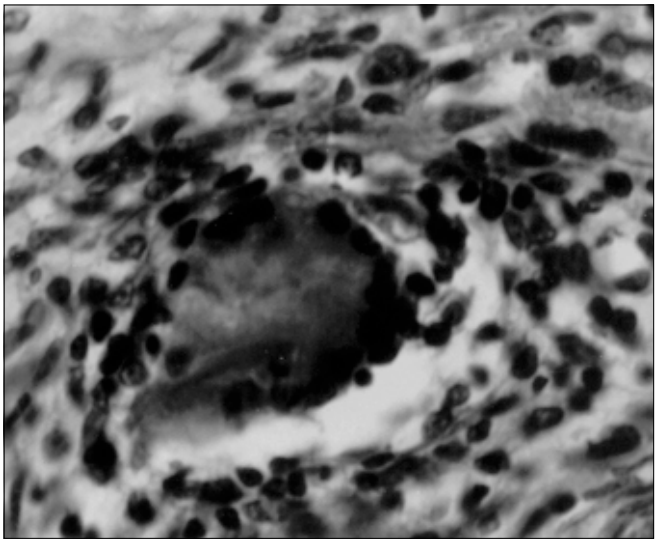

Fig. 3. Giant multinuclear cell in capsule. Hematoxylin and eosin staining, ×200.

number of vessels decreased to 1-3 per VF compared to previous term, which reflected cicatrix maturation. Solitary macrophage aggregates in the cicatrix probably reflect ongoing resorption of the cicatricial tissue.

Hence, blood flow at the site of MW treatment was arrested because of vessel destruction and impairment of blood rheology. Clear-cut demarcation of the necrotic focus was observed after 24 h; the focus was surrounded with the demarcation zone and separated from the liver parenchyma and abdominal cavity. After 7 days the necrotic focus was encapsulated and its zonal structure (capsule, demarcation zone, and detritus zone surrounded by them) remained unchanged for up to 60 days. Resorption of cell debris and maturation of the capsule took about 3 months. Because of involvement of giant multinuclear cells in this process and the formation of granulomas, this tissue reaction to detritus formed after MW destruction was similar to the reaction to a foreign body. After 4 months the focus of MW destruction was completely replaced with fine cicatrix consisting of mature connective tissue.

It is noteworthy that treatment of extensive hemangiomas of critical anatomical location is a complex problem: surgical interventions are difficult and life-threatening because of the risk of massive hemorrhages [4,6], while cryodestruction, sclerosing therapy

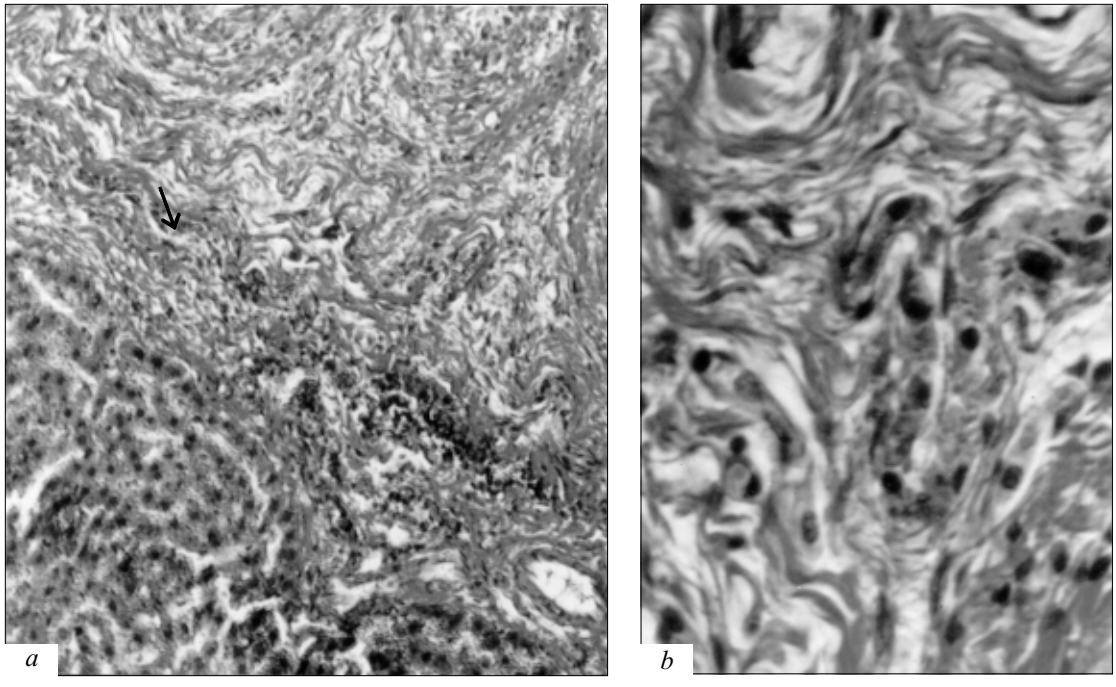

Fig. 4. Morphological changes at the site of MW destruction 120 days after exposure. Van Gieson staining, ×40 (*a*), ×200 (*b*). Necrotic focus is completely replaced with fine cicatrix (arrow).

and radiotherapy, diathermocoagulation are ineffective because they lead to just surface necrosis of the tumor [4-6]. Combined MW and cryogenic treatment is effective, but requires not only MW sources, but also special cryogenic devices [4].

Our data indicate that local MW destruction leads to the formation of extensive necrotic focus, the target of exposure being the vessels, and evolution of necrotic detritus eventuating in the formation of a fine cicatrix. All this means that MW irradiation in the destruction mode is an optimal methods for treating hemangiomas of complex anatomical location.

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