Comparative Morphology of Laser Lesion Sites in Parenchymal Organs (Liver, Kidney, Spleen)

V. A. Bychkovskikh, I. Ya. Bondarevsky, and L. V. Astakhova*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 739-741, May, 2012
Original article submitted February 28, 2011

The inflammatory and reparative processes in the parenchymal wounds after laser coagulation are characterized by predominance of proliferative reaction. Injury foci are always spatially separated from the intact tissue. Coagulation necrosis and thrombosis lead to suppression of the exudative component of inflammation in sites of laser exposures in the liver, kidneys, and spleen. Early macrophage response stimulates proliferation of fibroblasts and formation of the fine connective tissue scar within 14 days.

Key Words: *live; kidney; spleen; inflammation; laser*

The wound healing process is a dynamic self-regulating system with a stereotypical kinetics response: damage–mediator and microcirculatory response–exudation and migration of cells from the vessels–cleansing from the degradation products–proliferation of fibroblasts and vessel growth–collagen fibrillogenesis–maturation and transformation of the granulation tissue into the fibrous tissue–scar remodeling and involution [1]. The above universal inflammatory and reparative response does not depend on the damaging factors, but has some features associated with the type of damaging factor as well as with the structure and function of the operated organ [5,7].

Here we studied the features of inflammatory and reparative responses in operated parenchymal organs (liver, kidney, spleen) with the use of high-intensity laser radiation in the near infrared range.

MATERIALS AND METHODS

Experiments were carried out on 40 mongrel dogs. We studied the effect of high-intensity laser radiation generated by Sharplan 6020 diode laser (wavelength 805 nm, power 10 W) on the tissue of parenchymatous organs during resection of the liver, kidneys, and spleen carried out with quartz monofilament fiber. Fragments of standard size and location were excised from liver lobe, kidneys, and spleen pole. The exposure was performed in a pulsed mode for 10-20 sec. The organs were examined on days 1, 3, 5, 7, 10, 15, 21, 30, and 60 after resection. The samples were fixed in 10% neutral formalin and paraffin blocks were prepared. The sections were stained with hematoxylin and eosin, by the methods of Weigert and van Gieson, with alcian blue, and with Schiff reagent.

Statistical significance of the data was assessed by Student's *t* test using BIOSTAT software [3].

RESULTS

The comparative histological study on the sites of impact along with the research of the dynamics of reparative compensatory processes seemed to be interesting. The most pronounced reactive and alterative changes in sites exposed to laser radiation and in the surrounding tissues were observed on day 1 after resection.

Zones of laser photocoagulation had a number of similar signs; clear boundaries, the presence of several zones in all the foci, sharp boundaries between the zones without smooth transition from one zone to another.

Five zones were identified in all operated organs at the site of laser impact, namely: zone of charring,

Chelyabinsk State Medical Academy, Russian Ministry of Health; *Chelyabinsk State Institute of Laser Surgery, Russia. ***Address for correspondence:*** bond_il@mail.ru. I. Ya. Bondarevsky

zone of coagulation, zone of necrosis, zone of acute circulatory disorders and edema, and zone of degenerative changes.

Charring (carbonization) area was presented by yellow-brown and black particles overlaying the resected surface. Immediately under these overlays, a strip of tissue of varying width was detected with numerous small cavities formed by elongated disconnected cells.

The zone of coagulation was of greatest interest, because the processes occurring in the tissue during laser irradiation cannot be called truly necrotic.

Necrosis development takes a certain time from the pathological impact and exhibits a clear temporal dynamics. In a tissue layer located just below the site of carbonization, all cells and their nuclei were clearly seen, blood vessels were filled with compact or loosely lying erythrocytes with clear contours and scanty uniformly distributed white blood cells. No changes (vascular reaction, changes in intravascular blood rheology, edema, cellular response, *etc*.) were found either in the first minutes and hours or after 1 day after the impact. Such static picture persisted over 3 days after laser resection in approximately 30% cases (Fig. 1).

We also noted changes in the nucleus shape in adventitial cells located at the boundary with the deeper tissue layers. The nuclei of adventitial cells were stretched, became twisted, and were reoriented perpendicular to the surface of the organ capsule.

The next zone was an area of coagulation necrosis with intrinsic morphological temporal changes.

The zone of coagulation necrosis was separated from the parenchyma with a zone of acute circulatory disorders and edema, very narrow in all organs (2-4 rows of parenchymal cells). Paretic plethora of the capillaries as well as small and medium-sized veins with erythrostasis and slugged erythrocytes was found there. In some vessels, plasma was separated formed elements, and vascular walls were swollen and plasmatized. In rare cases, erythrocyte thrombi were detected in medium and small blood vessels. Later, the major reactive and cellular reparative processes occurred in this zone.

The zone of degenerative changes also did not occupy a large area and was presented mainly by different types of protein degeneration of parenchymal cells and in some cases, mild mesenchymal degeneration. Degenerative changes were transient and subsided disappeared within 21 days.

In general, it should be noted that coagulation necrosis and thrombosis lead to decreased the intensity of inflammation at the sites of laser impact due to reduction of exudative component of inflammation. Marked macrophage proliferation characteristic

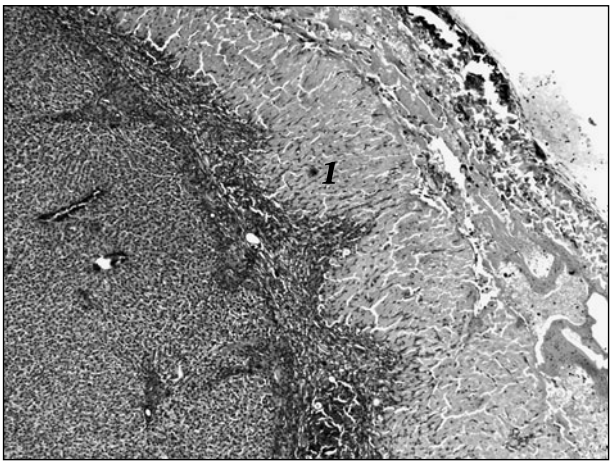

Fig. 1. The zone of laser resection in the liver (7 days after surgery). "Static picture" (*1*) at the site of primary laser injury of the parenchyma is delimited with a cellular fibrous shaft of the intact parenchyma. Carbonized masses and small cavities overlay the resected surface. Hematoxylin and eosin staining, ×50.

to aseptic inflammation (due to thermal effects) promoted fibroblast proliferation and associated collagen production as well as formation of the connective tissue [2,6].

Along with the above common morphological changes, foci of laser irradiation displayed organ-specific features [4,5]. Thus, in liver and kidney coagulation zone was filled with compact or loosely lying fibrin fibers with few neutrophils lying in their loops. Neutrophil response was detected in these organs on day 7 and subsided to day 15 (Fig. 2). Macrophages were reported on day 1, their amount was increased and peaked up to day 15, and again decreased up to day 30. Single fibroblasts appeared on day 1, and then their number increased up to day 15 followed by decrease through transformation into fibrocytes and reduction.

Study of the specific surface areas of connective tissue fibers after laser resections showed the formation of collagen and elastic fibers on day 3, increase in their surface area on day 15 and its reduction within 30 days due to contraction, convergence of fibers and their partial reduction. The study of the specific surface area of the vascular network revealed that neoangiogenesis started at day 3, then the number of vessels increased and reached a maximum up to days 15-21, and their partial reduction occurred up to day 30. In addition, multiple dark-purple clumps (insoluble calcium salts) were precipitated in the cytoplasm of the capsule epithelium on the border of the focus of coagulation necrosis and zone of dystrophic changes in the kidney.

In the spleen single thin fibrin fibers and a small amount of uniformly distributed white blood cells against the background of the compact mass of red

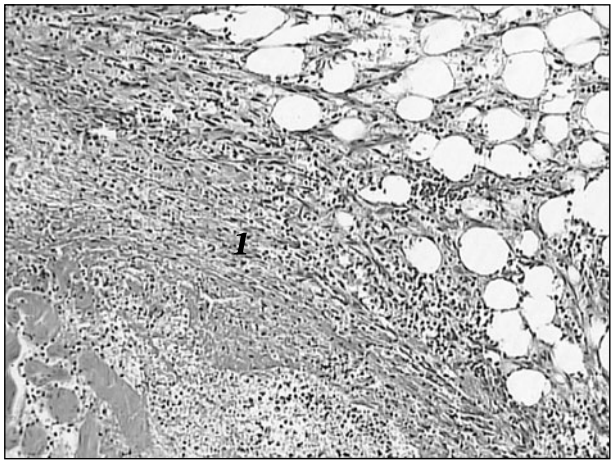

Fig. 2. The zone of laser resection in the kidney (7 days after surgery). Forming cell-fiber shaft in the perifocal zone (*1*) with areas of fibroblast proliferation and foci of macrophage, neutrophil, and lymphocyte infiltration. Hematoxylin and eosin staining, ×200.

blood cells were found. On day 1 after surgery the focus of necrosis was clearly separated from intact tissues with an inflammatory shaft. The number of fibroblasts that appeared on day 3 gradually increased, dominating in the sections on days 5-7 and reaching a peak by the end of week 3 after surgery. Lymphocytes were observed in the focus from day 3, their number piaked on days 7-10. Macrophages appeared on day 1, their content reached a maximum by day 5 and was zero in two weeks. Collagen fibers were reported in the border area starting from day 3. Their number progressively increased reaching a peak on day 21. The area of the vascular network demonstrated similar dynamics.

Thus, inflammatory reaction in the liver, kidney, and spleen after high-intensity laser irradiation run a similar course with a clear-cut predominance of the proliferative phase, which contributes to the early scar formation. Exudative changes were moderately expressed and completely ceased by day 15 after surgery. Along with common effects of laser irradiation on parenchymal organs, specific changes were detected in each due to specific organ functions and peculiarities of blood supply and metabolism in it.

REFERENCES

1. D. A. Ilyin, I. V. Maiborodin, *Khirurgiya*, No. 11, 70-72 (2002).
2. S. D. Pletnev, *Lasers in Clinical Medicine* [in Russian], Moscow (1996).
3. O. Yu. Rebrova, *Statistical Analysis of Medical Data* [in Russian], Moscow (2002).
4. R. M. Safarov and Yu. V. Kudryavtsev, *Urology and Nephrology*, No. 6, 14-16 (1996).
5. H. Mecke, M. Schunke, S. Schnaidt, *et al*., *Res. Exp. Med*., **191**, No. 1, 37-45 (1991).
6. D. Schuppan, M. Ruehl, R. Somasundaram, and E. G. Hahn, *Semin. Liver Dis*., **21**, No. 3, 351-372 (2001).
7. K. Taari , J. O. Salo, L. Kivisaari, *et al*., *Urol. Res*., **21**, No. 4, 301-304 (1993).