EFFECT OF LOW-INTENSITY INFRARED LASER RADIATION ON ULTRASTRUCTURE AND PROLIFERATION OF LIVER CELLS IN EXPERIMENTAL HEPATITIS AND CIRRHOSIS

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The expanding use of low-intensity laser radiation (LILR) in different fields of medicine has aroused interest in the study of morphological aspects of the action of LILR on cells, tissues, and organs. The clinical use of laser therapy has often preceded fundamental research into the action of LILR. It has now been shown that the therapeutic effect of LILR, such as the helium-neon laser, or lasers on nitrogen and copper vapor, is based on an increase in proliferative activity, more rapid differentiation of the cells, and a reduction in volume of the juxtamural microflora [2, 3, 8]. LILR in the infrared band (λ = 890 nm) from a semiconductor arsenide-gallium laser (AGL) of the "Uzor" type [3-5, 8] is widely used in clinical practice at the present time. Experimental research has shown that AGL, more than other types of LILR, induces intensification of the microcirculation [4, 5]. The quite high penetrating power of LILR in the infrared band enables the "Uzor" to be used effectively percutaneously [5, 7] in the laser treatment of the digestive organs and, in particular, of the liver, which possesses great powers of regeneration even under pathological conditions [6]. However, there has been no investigation of the effect of AGL on the ultrastructure and proliferation of cells of the normal liver or in hepatitis and cirrhosis, which will provide a morphological justification for the targeted use of AGL in liver pathology.

METHODS

The ultrastructure and proliferation of liver hepatocytes of 54 Wistar rats weighing 120-140 g was studied. The anterior abdominal wall of the rats in the region of the right hypochondrion and epigastrum, previously shaved, was irradiated by AGL (frequency 80 Hz, exposure 128 sec, power of radiation 3 mW), in 3, 5, or 7 sessions with an interval of 24 h between sessions. The following groups were used: I) 18 rats without liver pathology (9 — control, 9 — with irradiation by LILR). Toxic hepatitis was induced by subcutaneous injection of a 50% solution of $CaCl_4$ in cottonseed oil in a dose of 0.5 cm^3/100 g body weight, 10 times with an interval of 24 h between injections. III) 18 Rats with experimental cirrhosis of the liver (9 — control, 9 with irradiation by LILR), induced by the method of von Tsironis and Paracharalampous [9]. The animals were killed by decapitation 3 days after the last irradiation. For autoradiography, ^{3}H-thymidine was injected into the animals intraperitoneally 1 and 24 h before decapitation in a dose of 18.5 μBq/g body weight.

Tissue was fixed in 2.5% glutaraldehyde solution in phosphate buffer. Paraffin sections were dewaxed and covered with type M emulsion. The index of labeled nuclei (ILN) was determined by counting 10,000 hepatocytes. Semithin Epon-Araldite sections were stained with methylene blue and fuchsine, and ultrathin sections, after double

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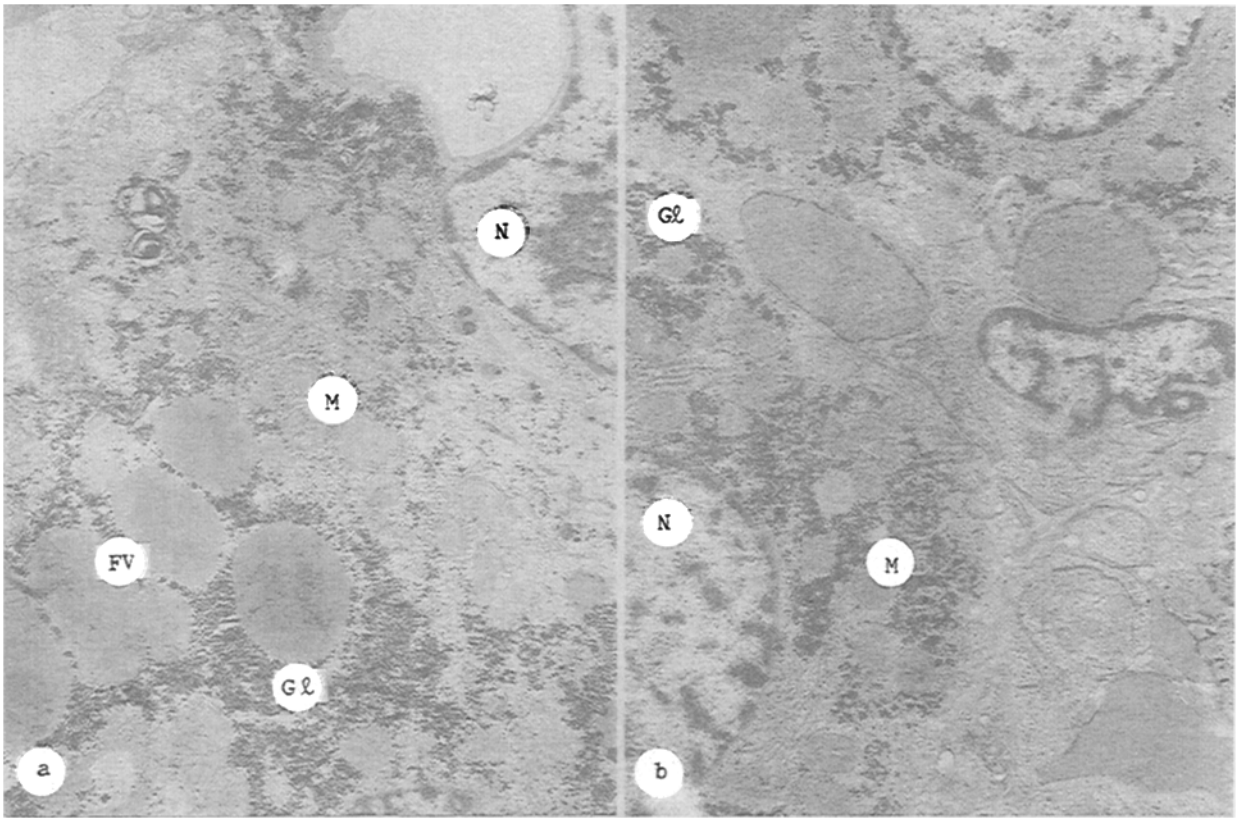

Fig. 1. Ultrastructure of hepatocytes in experimental toxic liver damage. a) Numerous lipid vacuoles, appearance of myelin corpuscles, reduction of volume of mitochondria and rough endoplasmic reticulum of hepatocytes. TEM, 5000×. b) Decrease in size of lipic vacuoles, increase in volume of mitochondria, and of rough endoplasmic reticulum in liver cells after irradiation by arsenide—gallium laser. TEM, 5000×.

contrast staining, were studied on the Hitachi H-600 electron microscope. Stereomorphometry was undertaken on electron micrographs using standard magnification, with the aid of a grid [1].

AGL induced morphological changes in the liver indicative of activation of the microcirculatory bed. The Disse's spaces and the lumen of the sinusoidal capillaries were dilated, as also was the lumen of the other microvessels and the central veins. This was accompanied by an increase in density of the cytoplasmic structures, in the number and size of the microvilli on the surface of the hepatocytes and the number of cells lining the sinusoidal capillaries. In the hepatocytes the relative bulk density of the mitochondria and of structures connected with heterosynthesis increased. For instance, the relative volume of the mitochondria in the control was 50%, of the Golgi complex 1.5%, whereas in animals irradiated by the AGL it was 62 and 2.1%, respectively.

With some decrease in the relative fraction of the volume occupied by glycogen in the hepatocytes the density of the glycogen granules increased significantly. The dimensions of the nucleoli and density of the chromatin increased, as also did the number of pores in the nuclear membrane. ILN of the hepatocytes of animals irradiated by AGL increased from 0.02 to 0.028%.

The maximal increases in the relative bulk fraction of the intracellular structures and of ILN occurred after 5-7 sessions of laser therapy.

The morphological manifestations of experimental toxic liver damage consisted of edema and infiltration of the stroma by polymorphs, and edema of the hepatocytes, causing narrowing of the Disse's spaces. A varied degree of degeneration of the hepatocytes took place, from granular to fatty. Individual areas with necrotic hepatocytes, most frequently located in the center, were seen in the lobules. Ultrastructural investigations revealed vacuolation of profiles

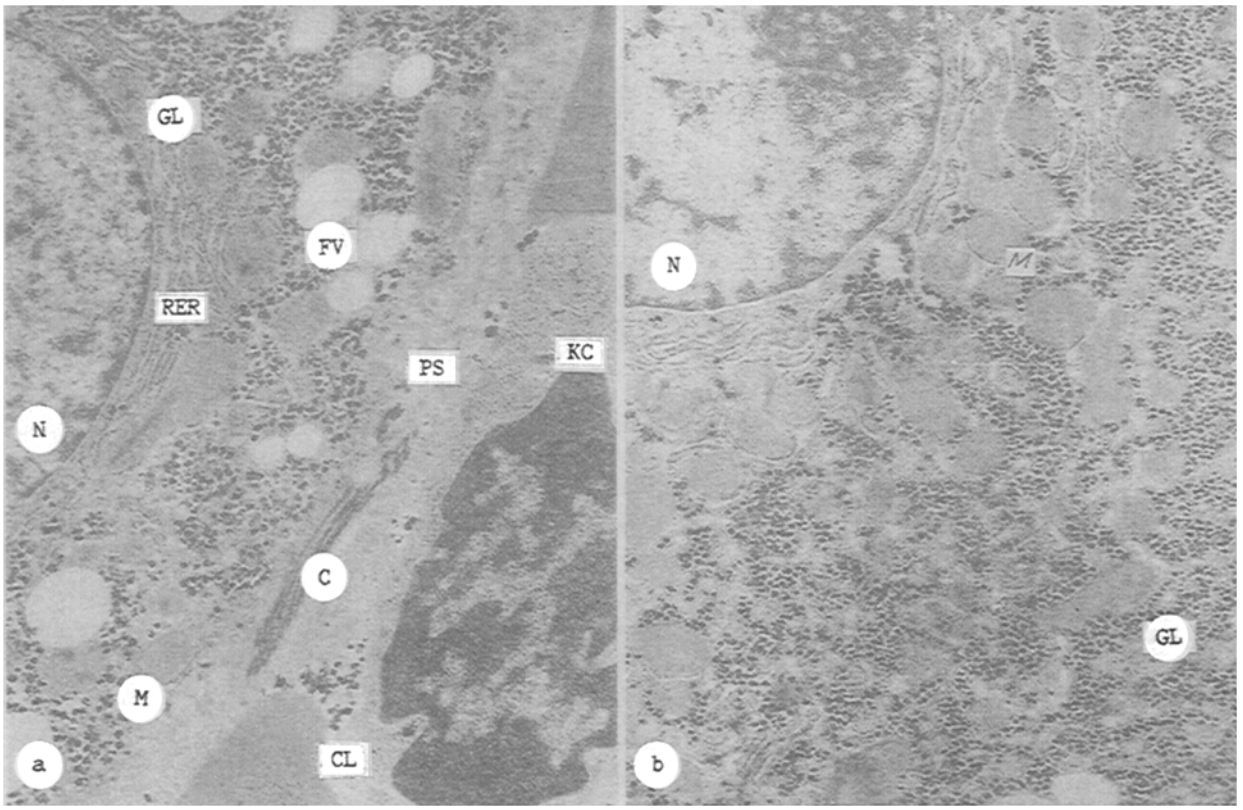

Fig. 2. Hepatocyte ultrastructure in experimental cirrhosis of the liver: a) reduction in volume of intracellular structures of hepatocytes, collagen fibrils in perisinusoidal spaces. TEM, 8000×. b) Increase in volume of intracellular structures of hepatocytes after irradiation by arsenide—gallium laser. TEM, 8000×. Here and in Fig. 1: N) nucleus; M) mitochondria; Gl) glycogen; RER) rough endoplasmic reticulum; FV) fat vacuoles; C) collagen fibers; KC) Kupffer cell (stellate reticuloendotheliocyte); PS) perisinusoidal space (of Disse); CL) lumen of sinusoidal capillary.

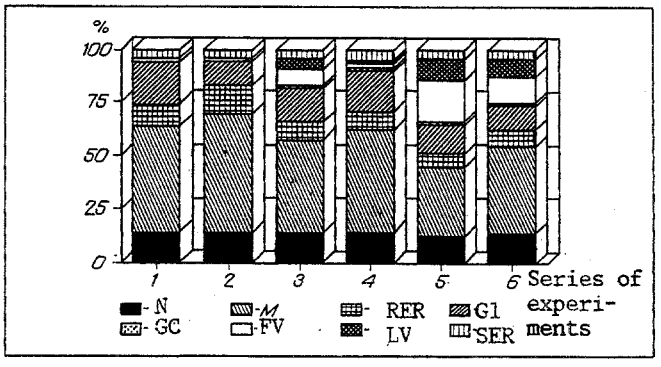

Fig. 3. Increase in relative bulk fraction of cytoplasmic structures maintaining energy processes and intracellular protein synthesis under the influence of AGL.

of the rough endoplasmic reticulum of the hepatocytes, swelling of the mitochondria, a decrease in the number of glycogen granules, translucency of the cytoplasm of the liver cells, an increase in size of various cytoplasmic corpuscles and vacuoles, and the appearance of large lipid drops (Fig. 1a).

Macroscopically, experimental cirrhosis of the liver can be classed as of the mixed type. Changes in the liver, besides those observed in toxic hepatitis, also included proliferation of connective tissue. Ultrastructurally, bundles of collagen fibers were identified in the interstices and in the Disse's spaces (Fig. 2a), with the presence of numerous fibroblasts with intracellular structures, indicating intensification of collagen formation.

Irradiation of the liver by AGL, both in toxic hepatitis and in cirrhosis of the liver, caused stereotyped changes manifested as reduction of edema and widening of Disse's spaces and of the lumen of the sinusoidal capillaries. Manifestations of granular, vacuolar, and fatty degeneration of the hepatocytes were reduced. This is accompanied by a decrease in volume of the collagen fibers in Disse's space. ILN rose from 0.73% in cirrhosis and 0.38% in hepatitis without irradiation by AGL to 0.98 and 0.51%, respectively, in the irradiated groups. On the ultrastructural level, the effect of AGL on the hepatocytes in cirrhosis and hepatitis was manifested as an increase in the relative bulk fraction of the cytoplasmic structures responsible for maintaining energy processes (mitochonria, glycogen) and intracellular protein synthesis (rough endoplasmic reticulum, Golgi complex, Fig. 3). The nucleoli became larger and the number of nuclear pores was increased. The volume and number of lipid drops in the hepatocytes and the number of various intracellular vacuoles, lysosomes, and other formations, increasing in toxic hepatitis and cirrhosis, were reduced (Figs. 1b, 2b, 3).

Thus these investigations showed that irradiation of the liver in toxic hepatitis and cirrhosis reduces the severity of the pathological changes; the morphological manifestations of improvement of the microcirculation, reduction of the edema and degeneration of the hepatocytes, and enlargement of the cytoplasmic structures responsible for maintaining energy metabolism and synthetic processes, may serve as structural and experimental basis for the use of low-intensity laser therapy in the infrared band in the combined treatment of hepatitis and cirrhosis of the liver.

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