MORPHOLOGY AND PHYSIOLOGY OF WOUNDS INFLICTED BY AN ULTRASONIC DESTRUCTOR

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Ultrasonic destructors are nowadays used in laryngo-otorhinology, stomatology, and various branches of oncology and surgery [5, 6, 9]. It must be admitted, however, that the morphologic picture and mechanisms of tissue damage by these instruments have been incompletely studied [3, 8]. We know that an adequate approach to the study of surgical wounds and, in particular, wounds inflicted by various types of lasers, is a combination of different methods of investigation [1, 2, 8]. The aim of this investigation was to study the particular features of the ultrastructural and enzyme histochemical changes in a wound inflicted by an ultrasonic destructor, and also to undertake a biochemical evaluation of the resistance of the damaged tissue to proteolysis.

EXPERIMENTAL METHOD

Experiments were carried out with the "EstoRex" surgical ultrasonic destructor, manufactured by the Experimental Department of Tartu University, and working on an oscillation frequency of 60 kHz, with a power of 6 W, and a vibration amplitude at the working end of the destructor of 15 μ. A linear incision was made on the anterior surface of the liver of anesthetized rats, at a speed of about 20 mm/sec. Tissue samples were taken 5-7 sec and 24 h after the incision was made, and processed for electron-histochemical detection of glucose-6-phosphatase (GP) [10]. To assess proteolytic resistance of the wound material from the rat liver, strips of tissue 2 mm thick were excised, treated with the destructor for 45 sec, and then incubated for 24 h in a mixture of trypsin and chymotrypsin, followed by analysis of the proteolysis products [1]. Complete proteolysis of a suspension of washed residues of tissue homogenates also was carried out with cathepsin L (1 mg/ml in 0.1 M acetate buffer, pH 5.1, with the addition of 0.05 ml of 40 mM mercaptoethanol) for 60 h at 25°C. The remaining material was solubilized in 5 N KOH.

EXPERIMENTAL RESULTS

The whole region of ultrastructural lesions of liver tissue around the wound was conventionally divided into three adjacent zones: a zone of inactivation (GP of the hepatocytes was inhibited in the presence of deep lesions of the cells and their organelles), a transition zone (GP inhibited partially with lesions of many organelles), and an active zone (normal GP, with lesions of individual organelles.

Immediately after the incision the zone of inactivation consisted of debris, mainly vesicles of different sizes and electron density, single severely damaged mitochondria and peroxysomes, and fragments of deformed membranes (Fig. 1a). In the proximal (nearest to the preceding zone) region of the transition zone the general organization of the cells remained intact. However, in some cells, against the background of edema of mitochondria and peroxysomes, vesiculation of fragments of the smooth and rough endoplasmic reticulum (SER and RER) were observed, a small proportion of them exhibiting GP activity. Histochemical reaction products in these cells sometimes diffused into the cytosol (Fig. 1b). In the

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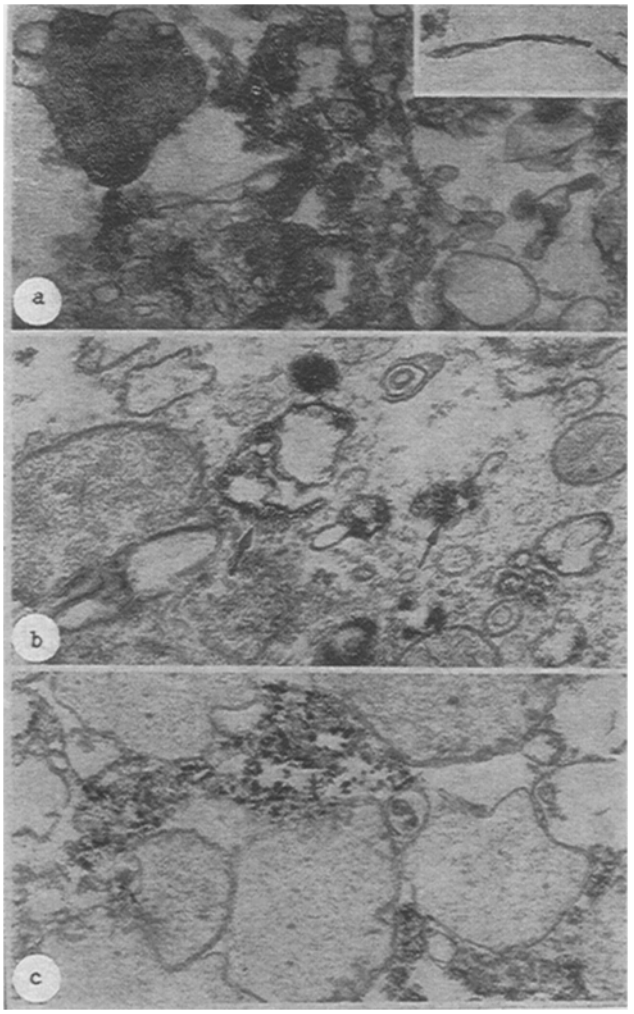

Fig. 1. Lesions of hepatocytes 5-7 sec after incision made with ultrasonic destructor. a) Zone of inactivation. Cell debris consists of vesicles of different sizes and electron density, severely damaged peroxysomes and mitochondria, and also single fragments of deformed membranes (insert), 82,200×; b) proximal region of transition zone. Vesiculation of SER and RER, edema of mitochondria and peroxysomes. Diffusion of products of reaction for GP in cytosol (arrow). 82,200×. c) In some hepatocytes marked widening of cisterns of SER and RER can be seen. Precipitates of cerium phosphate have the appearance of tiny granules. 30,200×.

other cells there was marked widening of the cisterns of the SER and RER but without any vesiculation. Precipitates of cerium phosphate filled their lumen, not completely as in intact cells, but in the form of tiny granules (Fig. 1c). Distally the predominant picture was one of vesiculation of SER and RER, while the ultrastructure of the other organelles was relatively intact (Fig. 2a). In many vesicles GP activity was found. Here also could be seen hepatocytes with structurally preserved cisterns of their SER and RER, but with total or partial suppression of GP activity in them (Fig. 2b). It is interesting to note that in the last case, deposition of products of the reaction for GP in these cisterns alternated with regions free from enzyme activity. Characteristic features of damage to the cells in the active zone were deformation of cisterns of the SER and RER and also partial destruction of the plasmalemma (Fig. 2c). These changes were localized most frequently at the vascular pole of the hepatocytes. The dimensions of all zones of injury, especially active, increased appreciably 24 h after the experiment began. Intensive infiltration of cell debris with neutrophilic leukocytes and macrophages with signs of phagocytosis was observed. In cells of the transition zone profound dystrophic changes took place,

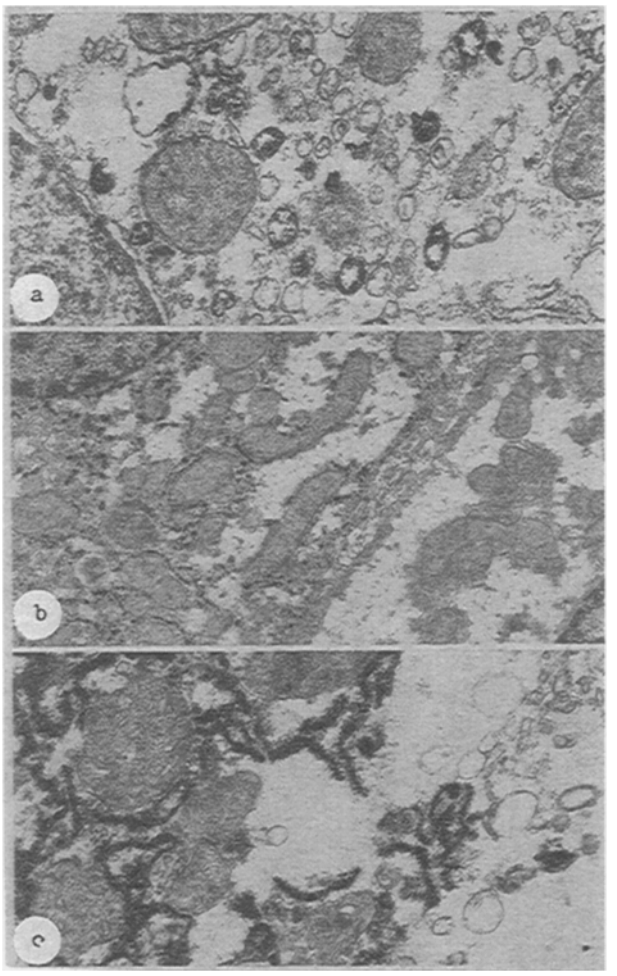

Fig. 2. Damage to hepatocytes: a) vesiculation of SER and RER can be seen in hepatocytes in distal part of transition zone, with preservation of GP activity in many vesicles, and with very small lesions of other organelles. 30,200×. b) Cells with structurally preserved cisterns of SER and RER, but with complete (hepatocyte on right of biliary capillary) or partial (to its left) suppression of GP activity. 11,200×. c) Active zone. Deformation of cisterns of RER and region of destroyed plasmalemma at vascular pole of cell. 30,200×.

leading to the formation of complex, closed cross sections of the RER and SER, often containing products of the reaction for GP. Hepatocytes of the active zone were in a state of fatty-degeneration (Fig. 3a), the principal feature of which was the presence not only of membrane structures at the periphery in many lipid droplets, but also fragments of the cytosol and organelles throughout the thickness of these drops (Fig. 3b).

The biochemical investigation showed that homogenates of sonicated liver tissue contained more TCA-soluble products than in the control (Table 1). As a result of complete proteolysis in a mixture of trypsin and chymotrypsin, and also in the case of lysis with cathepsin L, the concentration of these products increased considerably. Their release from the experimental samples, moreover, was either lower than (in a mixture of proteinases) or identical with that in the control (in a solution of cathepsin L). The concentration of products solubilized from the residue in 5 N KOH, on the other hand, was sharply reduced at the end of the experiment by comparison with initial levels. Incidentally, the amount of material resistant to proteolysis in the mixture of pancreatic proteinases was less in the experimental samples than in the controls, whereas in the case of cathepsin L it was the same.

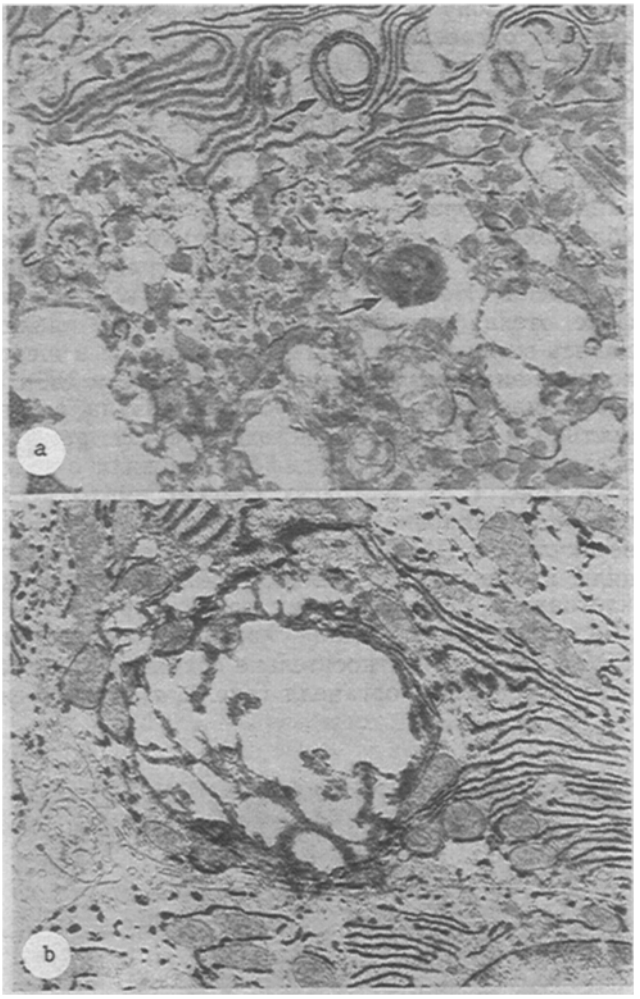

Fig. 3. Liver damage 24 h after incision made with ultrasonic destructor. Active zone. a) Progressive fatty degeneration and formation of autophagosomes (arrows). 11,100×, b) Large lipid drop containing fragments of cytosol and organelles in its substance. 11,100×.

The experiment showed that some of the mechanical energy of the ultrasound is transformed in biological objects into heat energy [6]. Nonthermal effects such as cavitation and viscous stresses [7, 9] have been studied less completely. Cavitation can be subdivided into stable, pulsation of which creates acoustic microflows and shear stresses, and collapsing, causing a sudden local rise of temperature and pressure at the moment of collapse. Viscous stresses in turn also include microflows and shear stresses, and in addition, radial pressure and other less important physical factors. The complex pattern of various types of cell lesions found in the present investigation is evidently the result of interaction between different effects of ultrasound, whose intensity decreases away from the wound surface into the thickness of the tissue. Analysis of activity of the thermolabile enzyme GP enabled only the thermal effect to be confidently distinguished. Identification and assessment of nonthermal effects of ultrasound under the experimental conditions used are possible only indirectly. One way of doing this is to compare the changes found with those observed after "purely thermal" action and, in particular, radiation of an infrared Nd:YAG laser [2]. Comparison in this way demonstrated the similarity of thermal inhibition of GP activity in the two cases. Meanwhile multiple ruptures of the membranes and vesiculation of SER and RER are probably the result of the combined action of mechanical factors generated by the destructor, namely cavitation and viscous stresses. It is interesting to note that vesiculation of SER and RER can be caused also by an ultraviolet excimer laser, in the action of which the photodynamic effect is predominant [8]. The molecular mechanisms of transformation of a planar membrane into a vesicular membrane still remain unexplained in both these cases.

TABLE 1. Content of Protein and Its Breakdown Products in Liver Samples Treated with Ultrasonic Destructor and Controls (M ± m)

Fraction of homogenate	Content of protein and its breakdown products (nmoles/Tyr/mg tissue) in TCA-soluble fraction (A) and TCA-insoluble fraction, solubilized with 5N KOH (B), at n = 3 for each point			
	control samples		experimental samples	
	A	B	A	B
Aqueous extract	57,5±0,9·	227,1±3,7	68,7±0,6·	243,2±6,9
Suspension of residue after proteolysis 24 h in mixture of proteinases	178,8±1,6··	14,9±0,9+	165,4±3,4··	8,8±0,7+
60 h with cathepsin L	131,0±1,3	16.7±0,5	121,2±9,0	16,8±1,0

Legend. Differences significant at $p < 0.05$, for (·, ··, +).

These investigations demonstrated inhibition of processes of autophagy and lysosomal degradation in the liver tissue of a rat after infliction of a wound by Nd:YAG laser, and a specially developed biological test with transplantation of a rabbit liver autograft subcutaneously in the concha auriculae revealed slowing of resorption of the irradiated tissue [2]. This tissue, as the biochemical investigation showed, contained material resistant to proteolysis, and this shed considerable light on the causes of the prolonged course of aseptic inflammation in wounds inflicted by an Nd:YAG laser [1]. The results suggest that denaturation of proteins in an ultrasonic wound was limited to conformational changes in the tertiary structure of the molecule. These findings suggest that an ultrasonic destructor can be regarded as a promising surgical instrument.

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