Structural and Metabolic Characteristics of Liver Acini in Dogs with Acute Cholecystitis

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Structural and metabolic changes in liver tissue in dogs with acute cholecystitis were studied by histological, histoenzymatic, and biochemical methods. It was found that the development of destructive inflammatory processes in the gallbladder wall induces morphological damage to the liver tissue, which correlates with metabolic disorders in this organ and leads to liver dysfunction.

Key Words: *liver acinus; dehydrogenases; cholecystitis*

Liver dysfunction in patients with acute cholecystitis is determined by many factors, *e.g.* infection and intoxication (especially, in destructive forms), concomitant diseases, mainly in elderly and senile patients, and surgical and anesthesiological stress [1,6,8,10]. The course and outcome of cholecystitis depend on morphological changes in the gallbladder [2,7]. The purpose of this study was comparison of structural and metabolic changes in the liver in various forms of acute cholecystitis in experimental animals.

MATERIALS AND METHODS

Thirty-six mongrel dogs with acute cholecystitis were examined. A modified experimental model of cholecystitis (proposed by V. S. Shevchenko in 1982) was used: catgut ligature was applied to the cystic duct and staphylococcal bacterial suspension was injected into the gallbladder through an insulin needle. The dogs were euthanized after 1, 3, 5, and 7 days. The control group (n=5) consisted of sham-operated animals. All experiments on animals were carried out in accordance with "Regulations of Animal Experiments".

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Liver fragments were collected from the right lateral lobe, embedded in paraffin, and stained with hematoxylin and eosin. Histochemical reactions were carried out on cryostat sections. Dehydrogenase activities (succinate, malate, glutamate, and lactate - SDH, MDH, GDH, and LDH, respectively) were measured using a Mecos-C image analysis system. Blood tests characterizing liver function (albumin, triglycerides, glucose and alanine aminotransferase and cholinesterase activities) were performed.

RESULTS

Morphological changes in the liver of dogs with acute experimental cholecystitis corresponded to the picture of nonspecific reactive hepatitis, which is in line with published data [3,4]. Catarrhal cholecystitis in the gallbladder wall developed on day 1 of the experiment. Morphological changes in the liver during this period were moderate microcirculatory disorders and hepatocyte damage mainly in the perivenular zones of acini, most pronounced in the gallbladder bed, and weak lymphohistiocytic infiltration of some portal tracts.

Microscopic picture on day 3 of the experiment was characterized by progressive inflammatory reaction in the gallbladder wall and phlegmonous cholecystitis. This was paralleled by aggravation of morphological changes in the liver parenchyma: more pro-

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nounced plethora of the sinusoids in the periventricular zone of the acini, development of reversible and irreversible changed in perivenular and intermediate hepatocytes (vacuolation of the cytoplasm, small-droplet fatty inclusions, small necrotic foci). Portal tracts near the gallbladder bed were unevenly edematous with weak lymphohistiocytic infiltration. The detected morphological changes in the liver tissue can be regarded as signs of lobular nonspecific reactive hepatitis.

On day 5 of the experiment phlegmonous ulcerative cholecystitis developed in the gallbladder wall. Morphological changes in the liver were characterized by progression of degenerative and necrotic processes in the perivenular and partially in the intermediary zone, weak inflammatory infiltration of the majority of portal tracts, which attests to lobular form of low activity nonspecific reactive hepatitis.

On day 7 phlegmonous gangrenous cholecystitis was observed in the gallbladder wall. Circulatory disorders in the liver became more pronounced. Portal vessels were plethoric with separation of the plasma and erythrocyte aggregation. The sinusoids of perivenular and partially intermediary zones were unevenly dilated and plethoric and contained erythrocyte aggregates and hemolyzed erythrocytes. Hemorrhages were seen in the perivenular zones of some acini. Perisinusoidal Disse spaces were dilated, Kupffer cells

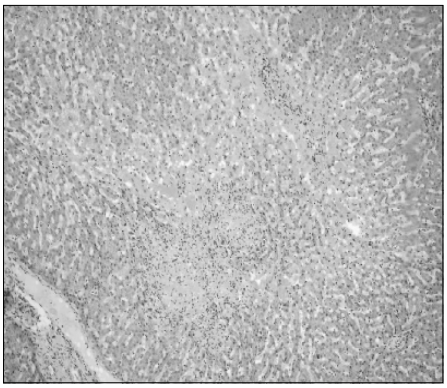

Fig. 1. Triangular necrosis. Day 7 of acute cholecystitis. Hematoxylin and eosin staining, ×100.

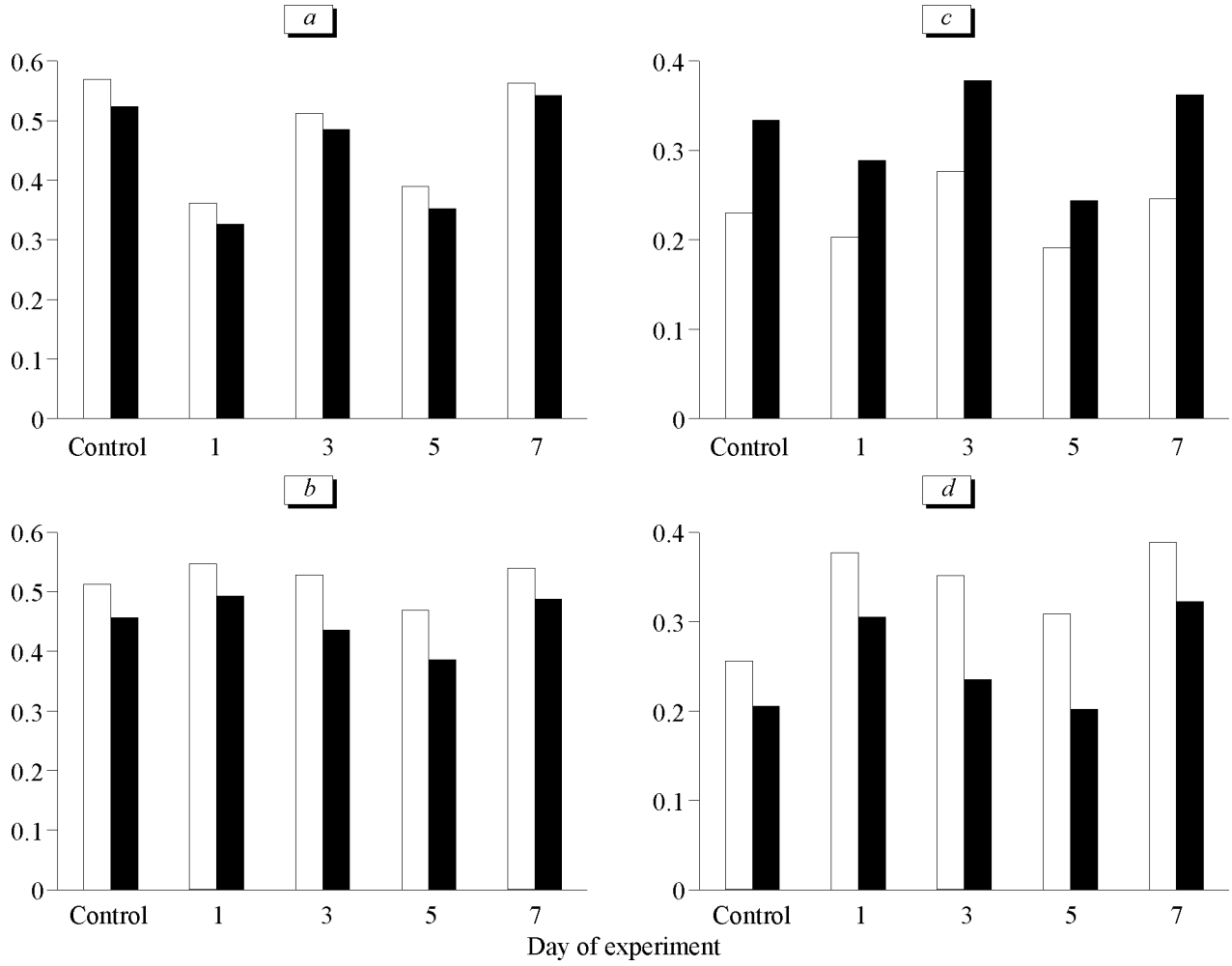

Fig. 2. Dehydrogenase activities in the liver of dogs with acute cholecystitis. a) LDH, b) SDH, c) GDH, d) MDH. Ordinate: optical density units. Light bars: 1st zone. Dark bars: 3rd zone.

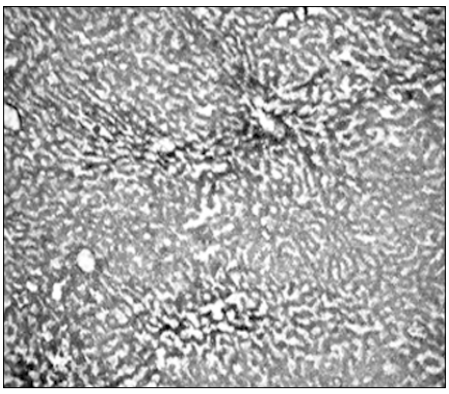

Fig. 3. Decreased SDH activity in the 3rd zone of the acinus. Day 3 of acute cholecystitis.

were enlarged with swollen cytoplasm. In 1 of 5 observations triangular necrotic hepatocytes were arranged along the periportal zones of the acini, in addition to hepatocyte degeneration in perivenular zones (Fig. 1). The cytoplasm of some hepatocytes contained dark brown lumpy incorporations (bile pigment). The portal tracts in the gallbladder bed were dilated, edematous, with diffuse inflammatory infiltration. Dissemination of inflammatory infiltration from the portal tracts into the periportal parenchyma with hepatocyte necrosis and impairment of the acinus structure was seen in 3 dogs. These morphological changes were identified as lobular form of moderately active nonspecific reactive hepatitis.

Histochemical study showed signs of stimulation of energy production in hepatocytes. Activation of the Krebs cycle reactions was observed. It sould be noted that that the morphological picture of granular cytoplasm of hepatocytes, observed as early as on day 1 of the experiment, can reflect not only degenerative changes, but also intensification of metabolic processes in cells [5].

When investigating the mechanisms of liver reaction to acute inflammatory destructive process in the gallbladder, we noted changed activities of liver enzymes, which can reflect the initial signs of damage. Histological analysis showed that activities of the key enzymes of Krebs cycle (SDH and MDH) gradually increased in parallel with aggravation of intoxication. The most pronounced increase in SDH and MDH activities on day 1 of the experiment probably attests to compensatory nature of this reaction. The most pronounced activation of MDH in the 3rd zone of the acinus (by 48.7% compared to the control, $p<0.05$) can be explained by more intense oxidation of Krebs cycle metabolites and involvement of this enzyme in the transport of redox equivalents between the mitochondria and cytoplasm.

Analysis of enzyme activities in hepatocyte revealed phasic changes of these parameters (Fig. 3). Activities of SDH and MDH decreased by day 3 of the experiment (Fig. 3), while LDH and GDH activities peaked at this term. Activation of GDH seemed to be caused by the compensatory coupling of protein catabolism and Krebs cycle. It is noteworthy that destructive forms of acute cholecystitis develop by day 3 of the experiment. High SDH activity during the first 3 days was more pronounced in the 3rd zone of the liver acinus. The peak of serum alanine aminotransferase activity on day 3 also confirms damage to the liver at this term. The drop of activities of all hepatic enzymes on day 5 of the experiment can be explained by progression of inflammatory and destructive changes in the gallbladder and exhaustion of the compensatory potential of the liver. The increase in serum LDH and GDH activities on day 5 reflects progressive reversible and irreversible damage to hepatocyte. This is also seen from high serum alanine aminotransferase activity on days 5 and 7 of the experiment.

Comparison of the dynamics of enzyme activities in hepatocytes and serum biochemical indexes revealed no linear correlations. We noted however that shifts in hepatocyte enzyme activities were paralleled by similar but slower changes in serum parameters.

According to our concept of periodical wave-like changes in liver metabolism, secondary changes in the sera can be expected on day 5 of the experiment. Indeed, GDH, LDH, triglyceride, and albumin activities peaked on day 5 of the experiment. We expected changes in the serum glucose concentrations. However, we revealed no linear dependence between the dynamics of hepatocyte SDH activity and blood glucose. Serum glucose level was the least variable parameter. The compensatory processes in the liver against the background of stable glucose level should be supported by other energy resources. A 33% decrease in serum triglyceride level on day 1 of the experiment, its increase by 96% by day 3 and by 222% by day 5 ($p<0.05$) are therefore understandable.

Decompensation following the first compensatory wave in the liver was paralleled by a significant decrease in serum triglyceride level on day 7 of the experiment. Changes in triglyceride level were accompanied by changes in serum protein concentrations. Hypoalbuminemia developed by day 3 of the experiment attested to disturbed protein synthesizing function of the liver. This is confirmed by simultaneous decrease in cholinesterase activity, because the synthesis of this enzyme is impaired [9]. Histological studies showed protein degeneration of hepatocytes primarily in the perivenular zones. The drop of GDH activity in the 3rd zone of liver acini (by 13.5% compared to the control, $p<0.05$) is determined by predominant location of the enzyme in the 3rd acinus zone. GDH activity increased by day 3 at the peak of

the first compensatory wave, which could result from activation of a shared components of protein catabolism and Krebs cycle.

Hence, the development of destructive inflammatory processes in the gallbladder wall induced morphological changed in the liver tissue correlating with disorders in its metabolism and leading to liver dysfunction.

REFERENCES

1. N. N. Gurin and K. V. Logunov, *Treatment of Acute Cholecystitis* [in Russian], St. Petersburg (1999).
2. V. A. Kozlov, D. V. Airapetov, A. G. Makarochkin, and A. Yu. Prokopov, *Ann. Khir. Gepatol.*, No. 2, 51-57 (2002).
3. O. D. Mishnev and A. I. Shchegolev, *Liver in Endotoxicosis* [in Russian], Moscow (2001).
4. S. D. Podymova, *Liver Diseases* [in Russian], Moscow (1998), pp. 369-372.
5. D. S. Sarkisov, *Essays on the History of Pathology* [in Russian], Moscow (1993).
6. A. P. Chadaev and A. S. Lyubskii, *Lechashchii Vrach*, No. 8, 46-48 (1999).
7. R. Ambrosio, L. Leone, C. Peppas, *et al.*, *Minerva Chir.*, **50**, 39-46 (1995).
8. T. Juvonen, H. Kivinemi, and O. Niemela, *Eur. J. Surg.*, **21**, 365-369 (1995).
9. E. Schmidt and F. W. Schmidt, *Clin. Biochem.*, **26**, 241-251 (1993).
10. A Singer, G. McCracken, and M. Henry, *Ann. Emerg. Med.*, **28**, 267-272 (1996).