BG Pulse Journal USA
ISSN:1947-2536 http://www.bgpulseusa.com/index.php/bg
BG PULSE JOURNAL

PATHOHISTOLOGICAL CHANGES IN THE LIVER AND KIDNEYS OF WHITE RATS UNDER EXPERIMENTAL ETHANOL EXPOSURE

Qurbonova S.O., group 230, General Medicine
Tashkent State Medical University, Tashkent, Uzbekistan
Department: Anatomy, Histology, Pathological Anatomy
Scientific advisor: Associate Professor **Dusmetov A.T.**

Doi: https://doi.org/10.5281/zenodo.21997719

Abstract: The aim of the present study was to investigate the pathohistological changes in the liver and kidneys of white rats under experimental alcohol intoxication of varying duration, as well as to analyze the morphological parallels between the lesions of these two organs. The experiment used white male rats subjected to daily intragastric administration of a 40% ethanol solution at a dose of 5 g/kg body weight for 30, 60, and 90 days, along with intact control animals. It was established that chronic ethanol exposure causes, in the liver, the sequential development of fatty degeneration of hepatocytes, focal necrosis, activation of Kupffer cells, and lymphohistiocytic infiltration of the portal tracts, and, in the kidneys, vacuolar degeneration and necrosis of the proximal tubular epithelium, congestion of the cortical vessels, and initial signs of glomerulosclerosis. The severity of the morphological changes was directly dependent on the duration of alcohol intoxication.

Keywords: ethanol, alcohol intoxication, hepatic fatty degeneration, hepatocyte necrosis, nephropathy, tubular degeneration, experiment, white rats

Introduction

Relevance: Alcohol abuse remains one of the most significant medical and social problems of our time, since chronic alcohol intoxication leads to damage of virtually all body systems and occupies a leading place among the preventable causes of morbidity and mortality. According to the World Health Organization, alcohol consumption is annually responsible for a substantial proportion of cases of liver cirrhosis, chronic kidney disease, and cardiovascular disease, and the economic and demographic burden of alcohol-associated pathology continues to grow [1,4].

The liver is the main target organ in alcohol intoxication, since it is in the hepatocytes that the main metabolism of ethanol occurs, with the formation of acetaldehyde — a highly toxic compound that initiates oxidative stress, mitochondrial damage, and the triggering of apoptosis and cell necrosis [2,7,8]. Along with the liver, the kidneys, which are involved in the excretion of ethanol metabolites and are exposed to the direct and indirect toxic effects of both ethanol itself and the products of endotoxemia arising from damage to the intestinal barrier and liver, also undergo substantial changes [9,11]. Despite the considerable body of work devoted to alcoholic liver damage, the combined dynamics of pathohistological changes in the liver and kidneys at different durations of intoxication have not been sufficiently studied, and the available data are fragmentary in nature.

Understanding the sequence and interrelationship of structural changes in the liver and kidneys in experimental models makes it possible to clarify the pathogenetic mechanisms of alcoholic

damage to internal organs and to substantiate approaches to the early diagnosis, prevention, and pathogenetic correction of alcohol-induced visceropathies.

Aim and Objectives of the Study

Aim of the study: to investigate the nature and dynamics of pathohistological changes in the liver and kidneys of white rats under experimental chronic exposure to ethanol of varying duration.

In accordance with the aim of the study, the following objectives were set:

— to study the morphological changes in the liver of rats (hepatocytes, Kupffer cells, the vascular bed, portal tracts) on days 30, 60, and 90 of alcohol intoxication;

— to study the morphological changes in the kidneys of rats (the glomerular apparatus, tubular epithelium, interstitium, and vessels) at the same observation periods;

— to perform a quantitative morphometric analysis of the changes identified and to assess their dependence on the duration of ethanol exposure;

— to compare the nature and severity of pathohistological changes in the liver and kidneys in order to identify possible pathogenetic parallels.

Materials and Methods

The study was carried out on 60 white male Wistar rats weighing 180–220 g, kept under standard vivarium conditions with free access to water and food. The animals were divided into 4 groups of 15 animals each: a control group (receiving an equivalent volume of physiological saline) and three experimental groups, which were given daily intragastric administration of a 40% ethanol solution at a dose of 5 g/kg body weight for 30, 60, and 90 days, respectively. The animals were removed from the experiment by decapitation under ether anesthesia on the day following the last administration of ethanol, in accordance with the principles of humane treatment of laboratory animals.

Samples of liver and kidney tissue were fixed in 10% neutral buffered formalin and in Carnoy's fluid, dehydrated in ascending concentrations of alcohol, and embedded in paraffin according to standard procedure. Paraffin sections 5–6 μm thick were stained with hematoxylin and eosin to assess the general morphological picture; Van Gieson staining was also used to identify connective tissue elements, and Sudan III staining of frozen sections was used to verify lipid inclusions. For semithin sections prepared on an LKB-4800 ultramicrotome, methylene blue staining was used. Morphometric analysis was performed by light microscopy using an ocular grid; in each group, at least 10 fields of view per specimen were examined at ×400 magnification.

Quantitative assessment of liver changes was carried out by counting the proportion of hepatocytes showing signs of fatty degeneration and necrosis, the number of binucleate hepatocytes and Kupffer cells, and by measuring the diameter of the central veins of the lobules. When assessing the kidneys, the mean glomerular diameter, the proportion of glomeruli with sclerotic changes, and the proportion of tubular epithelium with vacuolar degeneration and necrosis were determined, and the severity of lymphohistiocytic infiltration of the interstitium and congestion of

the cortical vessels were assessed semi-quantitatively (on a scale of 0 to 3). Statistical processing of the results was performed by calculating the mean value and the standard error of the mean (M±m); the significance of differences between groups was assessed using Student's t-test, with differences considered statistically significant at $P<0.05$.

Results of the Study

Histological examination of the liver of control animals revealed a typical trabecular structure of the hepatic lobules, hepatocytes with clear borders, centrally located round nuclei, and moderately eosinophilic cytoplasm; the sinusoidal capillaries were moderately filled with blood, and the portal tracts showed no signs of cellular infiltration.

Already by day 30 of alcohol intoxication, the liver of the experimental animals showed the appearance of small-droplet fatty degeneration of hepatocytes, predominantly localized in the centrilobular and mid-zonal areas of the hepatic lobules. The cytoplasm of the affected hepatocytes contained numerous small lipid vacuoles, and the nuclei retained a central position. Moderate congestion of the sinusoids and initial activation of Kupffer cells were observed, their number being statistically significantly higher than control values.

By day 60 of ethanol exposure, the morphological picture of the liver was characterized by an increase in fatty degeneration, with the small-droplet form transitioning into a large-droplet form, in which a single large fat vacuole displaced the hepatocyte nucleus to the periphery of the cell. In the centrilobular zones, foci of balloon degeneration and isolated necrotic hepatocytes with pyknotic nuclei and eosinophilic homogenized cytoplasm (bodies resembling Mallory bodies) were identified. In the portal tracts and around the necrotic foci, moderately pronounced lymphohistiocytic infiltration with an admixture of neutrophilic granulocytes was determined. The sinusoidal capillaries were unevenly dilated and congested, and initial signs of perisinusoidal fibrosis were noted on Van Gieson staining.

By day 90 of the experiment, the changes became most pronounced: the proportion of hepatocytes with fatty degeneration reached nearly half of the lobular cell population, the number of necrotic foci increased, predominantly of centrilobular localization, with the formation of small inflammatory infiltrates in their place. Marked congestion of the central veins with an increase in their diameter, thickening and loosening of the walls of the portal tract vessels, and increasing periportal and perisinusoidal fibrosis were noted. The number of binucleate hepatocytes, reflecting the regenerative activity of the parenchyma, was statistically significantly reduced compared with the control and the 30-day group, indicating exhaustion of the compensatory-proliferative capacity of the liver under prolonged alcohol load.

The results of the morphometric analysis of the liver are presented in Table 1.

Table 1. Morphometric parameters of the liver of rats under experimental ethanol exposure.

Parameter	Control M±	Day 30 M±	Day 60 M±	Day 90 M±
Relative liver weight, %	3.21±0.06	3.45±0.08*	3.89±0.10**	4.12±0.11**

Parameter	Control M±	Day 30 M±	Day 60 M±	Day 90 M±
Hepatocytes with fatty degeneration, %	2.4±0.3	18.6±1.2**	34.7±2.1**	46.3±2.8**
Hepatocytes in necrosis, %	0.3±0.1	1.8±0.3*	5.4±0.6**	9.1±0.9**
Binucleate hepatocytes, %	4.1±0.4	3.2±0.3	2.0±0.3*	1.4±0.2**
Kupffer cells (count per field)	6.8±0.5	9.3±0.6*	13.2±0.8**	16.5±1.0**
Central vein diameter, μm	28.4±1.1	31.2±1.3	36.7±1.6**	41.5±1.8**

*Note: * P<0.05, ** P<0.001 compared with control*

Histological examination of the kidneys of control animals revealed a preserved nephron structure: rounded glomeruli with moderately pronounced mesangial cellularity, Bowman's capsule without signs of thickening, proximal and distal tubular epithelium of cuboidal and columnar shape with a clear brush border, and interstitium without signs of edema or cellular infiltration.

On day 30 of ethanol exposure, initial signs of vacuolar degeneration of the epithelium, predominantly of the proximal convoluted tubules, were found in the kidneys of the experimental animals: the cytoplasm of the cells became cloudy and swollen, with small vacuoles, and individual nuclei lost the clarity of their contours. The glomeruli were visually little different from the control, but moderate congestion of the cortical capillaries and isolated perivascular lymphohistiocytic accumulations were noted.

By day 60 of the experiment, vacuolar degeneration of the tubular epithelium became widespread, in places with features of balloon degeneration and desquamation of individual epithelial cells into the tubular lumen; in a number of tubules, small foci of epithelial necrosis with destruction of the basement membrane were identified. In the glomeruli, moderate expansion of the mesangial matrix, uneven congestion of the capillary loops, and thickening of individual sections of the capsule were noted. Lymphohistiocytic infiltration of the interstitium became more pronounced, predominantly in the peritubular zones of the cortex.

By day 90 of intoxication, the morphological changes in the kidneys reached their greatest severity: in a significant proportion of the animals, foci of necrosis of the proximal tubular epithelium with exposure of the basement membrane, pronounced vacuolar degeneration of the remaining epithelium, and desquamation of cells with the formation of cellular detritus in the tubular lumen were identified. The mean glomerular diameter was statistically significantly increased compared with control, which can be regarded as a manifestation of compensatory hyperfiltration against the background of a decrease in the functioning tubular mass; at the same time, some glomeruli showed initial signs of sclerosis of the capillary loops and thickening of the capsule, indicating the formation of chronic damage to the glomerular apparatus. The cortical

vessels were markedly congested, in places with features of stasis and diapedetic microhemorrhages into the interstitial tissue, and lymphohistiocytic infiltration was diffuse in character.

The results of the morphometric analysis of the kidneys are presented in Table 2.

Table 2. Morphometric parameters of the kidneys of rats under experimental ethanol exposure.

Parameter	Control M±	Day 30 M±	Day 60 M±	Day 90 M±
Mean glomerular diameter, μm	82.3±1.7	85.1±1.9	91.4±2.2*	97.8±2.5**
Glomeruli with signs of sclerosis, %	1.2±0.2	3.6±0.4*	8.9±0.7**	14.2±1.1**
Tubular epithelium with vacuolar degeneration, %	3.8±0.4	22.4±1.6**	39.7±2.3**	51.6±2.9**
Tubular epithelial necrosis, %	0.2±0.1	2.1±0.3*	6.3±0.6**	10.8±1.0**
Lymphohistiocytic infiltration of stroma (score, 0–3)	0.3±0.1	0.9±0.2*	1.6±0.2**	2.2±0.2**
Cortical vascular congestion (score, 0–3)	0.4±0.1	1.1±0.2*	1.9±0.2**	2.4±0.2**

*Note: * P<0.05, ** P<0.001 compared with control*

Discussion

The data obtained indicate that chronic alcohol intoxication causes a regular staged dynamic of pathohistological changes in the liver and kidneys of white rats, the severity of which increases in proportion to the duration of ethanol exposure. The development of fatty degeneration of hepatocytes in the early stages of the experiment is explained by impaired lipid metabolism due to a change in the NAD/NADH ratio during the oxidation of ethanol by alcohol dehydrogenase, which leads to inhibition of fatty acid β-oxidation and increased esterification of fatty acids into triglycerides [7,8,14]. The transition from small-droplet to large-droplet fatty degeneration and the appearance of necrotic foci by day 60–90 reflect the increasing damaging effect of acetaldehyde and reactive oxygen species on the membrane structures and mitochondrial apparatus of hepatocytes, which is consistent with current concepts of the pathogenesis of alcoholic liver disease [8,9,12].

Activation of Kupffer cells and increasing lymphohistiocytic infiltration of the portal tracts confirm the leading role of the inflammatory component in the progression of alcoholic liver

damage: damaged and dying hepatocytes, as well as products of endotoxemia caused by increased intestinal permeability against the background of chronic alcohol load, stimulate the release of proinflammatory cytokines, which contributes to the maintenance of chronic inflammation and the formation of perisinusoidal fibrosis [9,12,13]. The decrease in the proportion of binucleate hepatocytes at later stages of the experiment indicates exhaustion of the regenerative potential of the hepatic parenchyma, which represents an unfavorable prognostic sign in terms of the possibility of reversal of the morphological changes.

The morphological changes in the kidneys identified in this study are characterized by predominant damage to the proximal tubular epithelium, which may be associated both with the direct nephrotoxic effect of ethanol and acetaldehyde excreted in the urine, and with indirect damage due to systemic endotoxemia, oxidative stress, and impaired renal hemodynamics against the background of chronic alcohol intoxication [10,11,15]. The increasing congestion of the cortical vessels and perivascular lymphohistiocytic infiltration indicate the active involvement of the microcirculatory bed in the pathogenesis of alcoholic nephropathy, and the increase in mean glomerular diameter at later stages of the experiment reflects compensatory hyperfiltration developing in response to the progressive loss of functioning nephrons due to tubular necrosis.

Comparison of the dynamics of liver and kidney changes shows a pronounced parallelism in the timing of onset and severity of the pathological processes, which confirms the concept of the systemic nature of alcoholic damage to internal organs and allows chronic alcohol intoxication to be considered as a model of combined toxic hepatorenal dysfunction. The practical significance of the results obtained lies in substantiating the need for comprehensive assessment of the functional state of both the liver and kidneys when examining individuals with chronic alcohol intoxication, as well as in identifying potential morphological markers of the early stages of alcoholic damage to these organs.

Conclusions

1. Chronic intragastric administration of ethanol at a dose of 5 g/kg body weight causes progressive pathohistological changes in the liver of white rats, manifested by the sequential development of fatty degeneration of hepatocytes, focal necrosis, activation of Kupffer cells, and portal fibrosis, the severity of which increases from day 30 to day 90 of the experiment.

2. The decrease in the number of binucleate hepatocytes at later stages of intoxication indicates exhaustion of the regenerative-proliferative potential of the hepatic parenchyma under prolonged ethanol exposure.

3. In the kidneys of white rats, alcohol intoxication causes increasing vacuolar degeneration and focal necrosis of the proximal tubular epithelium, congestion of the cortical vessels, and initial signs of glomerular sclerosis, most pronounced by day 90 of the experiment.

4. The severity of pathohistological changes in the liver and kidneys is directly dependent on the duration of alcohol intoxication, as confirmed by the statistically significant increase in morphometric parameters of damage over the course of the experiment.

5. The parallelism identified in the timing and nature of liver and kidney damage indicates the systemic nature of the toxic effect of ethanol and substantiates the advisability of comprehensive

morphofunctional assessment of the hepatorenal system in experimental and clinical study of alcoholic disease.

References

1. Bondar T.P., Misetova E.N. Morphofunctional changes in the liver during chronic alcohol intoxication in an experimental study // Morphology. — 2019. — Vol. 155, No. 3. — P. 45–50.
2. Serov V.V., Lapish K. Morphological diagnosis of liver diseases. — Moscow: Meditsina, 2015. — 336 p.
3. Avtandilov G.G. Fundamentals of quantitative pathological anatomy. — Moscow: Meditsina, 2002. — 240 p.
4. Mukhin N.A., Moiseev V.S. Alcoholic disease: damage to internal organs in chronic alcohol intoxication. — Moscow: GEOTAR-Media, 2011. — 264 p.
5. Paukov V.S., Erokhin Yu.A. Pathological anatomy of alcoholism and alcoholic disease // Archive of Pathology. — 2018. — Vol. 80, No. 4. — P. 61–68.
6. Vetsheva N.N., Bykov V.L. Histological changes in the kidneys under experimental alcohol intoxication // Morphological Bulletin. — 2020. — Vol. 28, No. 2. — P. 33–39.
7. Louvet A., Mathurin P. Alcoholic liver disease: mechanisms of injury and targeted treatment. Nat Rev Gastroenterol Hepatol. 2015; 12(4):231–242.
8. Osna N.A., Donohue T.M., Kharbanda K.K. Alcoholic Liver Disease: Pathogenesis and Current Management. Alcohol Res. 2017; 38(2):147–161.
9. Latchoumycandane C., Nagy L.E., McIntyre T.M. Chronic ethanol ingestion induces oxidative kidney injury through taurine-inhibitable inflammation. Free Radic Biol Med. 2014; 69:403–416.
10. Gao B., Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology. 2011; 141(5):1572–1585.
11. Varga Z.V., Matyas C., Paloczi J., Pacher P. Alcohol Misuse and Kidney Injury: Epidemiological Evidence and Potential Mechanisms. Alcohol Res. 2017; 38(2):283–288.
12. Crabb D.W., Im G.Y., Szabo G., Mellinger J.L., Lucey M.R. Diagnosis and Treatment of Alcohol-Associated Liver Diseases: 2019 Practice Guidance. Hepatology. 2020; 71(1):306–333.
13. Rehm J., Samokhvalov A.V., Shield K.D. Global burden of alcoholic liver diseases. J Hepatol. 2013; 59(1):160–168.
14. Sozio M., Crabb D.W. Alcohol and lipid metabolism. Am J Physiol Endocrinol Metab. 2008; 295(1):E10–E16.
15. Tremasov M.Ya., Galyautdinova G.G. Pathomorphological criteria for assessing toxic damage to the liver and kidneys in an experiment // Veterinary Doctor. — 2016. — No. 5. — P. 12–17.