Nonalcoholic Steatohepatitis Animal Model, Induced by High-Fat Diet and Tetracycline

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Abstract

As a result of research it was succeeded to receive the model of non-alcoholic steatohepatitis which is characterized by obesity, violation of the structural organization, a fabric necrosis, formation of fibrosis of a liver. The developed model of non-alcoholic steatohepatitis allows to study also staging of development of a fatty desease of the liver which is characterized by formation of a steatogepatitis and fibrosis. In quality the inducing factors the high-fat diet and antibiotics were used. The offered NASH model can be used in the field of chronic experiments for an assessment of extent of violation of a functional condition of hepatobiliary system and for search of the effective pharmacotheurapeutichepatoprotective agents.

Keywords: liver, nonalcoholic steatohepatitis, high-fat diet, tetracycline

1. Introduction

Nonalcoholic steatohepatitis (NASH) is a liver disease, characterized by a histologic similarity with an alcoholic liver disease, which occurs in people who do not consume a significant quantity of alcohol (Bellentani*et. al., 2010*;. Musso*et. al., 2010; Torres et. al., 2012*) .

NASH is believed to be one of the most common explanations for abnormal liver chemistries in adults (Attar *et. al.,* 2013). According to several authors, NASH is the hepatic manifestation of metabolic syndrome that is characterized by steatosis, inflammation, and fibrosis, and may progress to cirrhosis and carcinoma (Chen, *et. al.2008*; Polesel *et. al.2009;*Yun *et. al., 2013*). Risk factors for NASH include obesity, type II diabetes, hyperlipidemia, total parenteral nutrition, and the use of certain drugs, includingantibiotics (Pries et. al., 2008; Praveen *et. al.,* 2012).

The identification of fatty liver on imaging studies supports the diagnosis of NASH, which can be established definitively by liver biopsy.

The latter also provides useful prognostic information since most patients with simple steatosis follow an indolent clinical course, whereas those with steatohepatitis, fibrosis, or cirrhosis are more likely to develop clinically significant complications of liver disease.

Diagnostic criteria of nonalcoholic steatohepatitis:

1) Moderate or severe fatty lobular degeneration and inflammation (lobular or portal) in the presence or absence of Mallory hyaline bodies, signs of fibrosis or cirrhosis (according to a study of liver biopsy material).

2) The absence of alcohol (consumption of <40 g of ethanol per week). Negative results of several randomized blood samples to determine the blood alcohol in the presence of the serum marker of alcohol - transferrin-containing no-sialic acids.

3) No evidence of infection with hepatitis B and C. (Williams, 2008; Fan et. al., 2007).

The most common deviation of the laboratory analysis is to increase the level of aminotransferases, wherein the ratio of ALT / AST usually less than 1. Sometimes increases the level of alkaline phosphatase and gamma glutamine transferase.

For further understanding of NASH, development and characterization of appropriate animal models with metabolic abnormalities is important.

Experimental models of NASH are widely used to study the mechanisms of formation, determine the role of various factors of the external and internal environment in the development of pathological processes that allow to make a detailed examination of the pathogenesis and to find means for rational therapeutic intervention and prevention.

The desired pathophysiological changes for experimental models of NASH include steatosis, intralobular inflammation, hepatocellular ballooning, and ideally perisinusoidal fibrosis (Takahashi et. al., 2012). Furthermore, these pathophysiological features should be accompanied by metabolic abnormalities such as obesity, insulin resistance, dyslipidemia, and altered adipokine profile (Murine et. al., 2013).

Current rodent models of fatty liver disease rely on strains that carry spontaneous mutations (ob/ob, db/db), genetic manipulations, or formulated diets (methionine and choline deficient diet, high-fat diet), yet none of these modelsaccurately reproduce the broad range of factors that contribute to the histological spectrum of human NAFLD and its sequel.

More recently, combinatorial use of diets with high proportions of fat, trans-fatty acids, oxidized lipoproteins, or high-fructose drinking water have resulted in patterns of liver injury closer to that observed in NASH, although aspects such as significant fibrogenesis and carcinogenesis are still lacking.

L.H. Tetri added a sedentary lifestyle to a diet rich in trans-fatty acids and high-fructose corn syrup for a 16-week period and found that mice developed glucose intolerance and hepatic steatosis and inflammation (Tetri*et al.*, 2008).

Based on the "two hit theory" (Day *et. al.*, 1998), we tried to develop a new rat model of NASH with metabolic disorders.

2. Material and Methods

2.1 Animals.

Research is conducted on 150 males of Wistar Albino rats at the age of 9 months. The animals were fed with standard pellet diet and water ad libitum. They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (30±2ºC). All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

2.2 Treatment Design

150 animals (Male Wistar Albino rats) were randomized and divided in to three groups of 50 animals in each group.

The first group (n=50) was control, it was formed from the rats which were kept on a standard vivarium diet.

The second group (n=50) consisted of the rats who were on the special hyper high-calorie hepatogenous diet sated with animal fats, within 45 days. We used the fatty loading with melted beef fat, which made about 20% of the general structure of a diet, increasing the general caloric content of a diet. In addition, or the 30th days of research 30mg/kg tetracycline was injected intraperitoneally once daily for 10 days.

The third group (n=50) was similar to the second, but experiment proceeded within 60 days.

Animals daily looked round, the forage palatability was considered. During experience there were no cases of a mortality and diseases of animals.

2.3 Weight Measurements

All rats were weighed in grams and naso-anal lengths in cm. The body mass index (BMI) was calculated at the end of the study period. Measurements were taken at the beginning and at the end of research in each group. At the end of experiment it was measured absolute (in grams) and relative mass of a liver of rats.

2.4 Biochemical Analyses

Level of glucose and activity of an alaninaminotransferase (ALT), aspartataminotransferase (AST), lactate

dehydrogenase (LDG) in serum of blood investigated by means of the biochemical StatFax3300 analyzer (USA) by means of sets of Spinreact firm (Austria).

2.5 Histopathological Analysis

A small portion of liver was taken and fixed in to 10% formaldehyde. After several treatments for dehydration in alcohol, sections having 5μm thickness were cut. Sections were were subjected to stain with hematoxylin and eosin and van Gieson'spirofuchsin stain on identification of collagenic fibers, then the histopathological analysis was carried.

2.6 Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 packed program.Data were presented as mean ± standard deviation unless noted as different. Difference between the control and experimental groups was analyzed using Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

3. Results

As a result of the conducted research it is revealed that the keeping of animals on a fatty diet with application of a tetracycline leads to change of a number of the studied weight parameters. In both experimental groups we noted an essential gain of body weight of animals – 58.4±4.8 g in the first experimental group and 103.4±6.57 g in the second while in control the increase in body weight in 90 days made 31.0±3.2 g.

The absolute mass of a liver concerning control also increased to 14.84±1.0 g for the 60th day of research, and to 18.90±1.1. on 90 day. The relative mass of organ made 4.76±0,15 and 5.31±0.50 respectively against 3.0±0,15 in control.

Body-weight ratio made 7.65±0.17 in control, 14.84±1.0 in the first experimental group and 18.9±1.1 in the second experimental group.
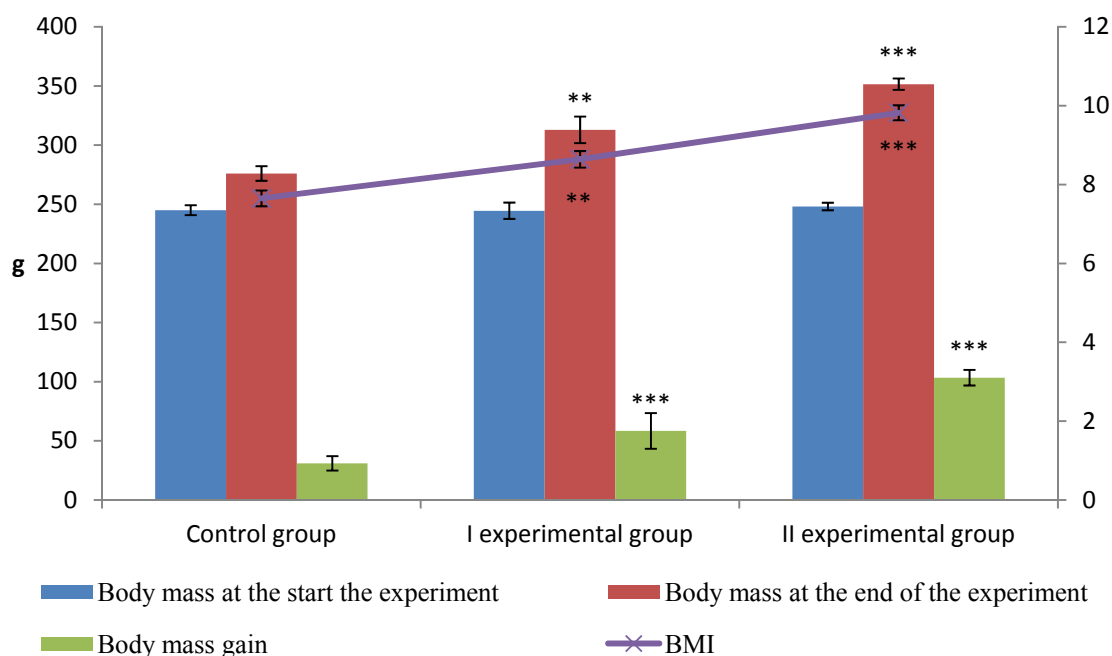

Figure 1. Weight and BMIof animals of controlandexperimental groups

As a result of biochemical researches increase of level of glucose in blood plasma from 5.44±0.10 mg/dL in control to 6.95±0.34 mg/dL in plasma of rats of the first experimental group and to 11.84±1.10 mg/dL in the second is established.

Similarly also the level of enzymes in blood of rats changes. ALT in control makes 50.21±3.70 U/L, in the first experimental group – 63.08±5.1 U/L and 170.5±21.1 U/L in the second.

Thus the level of AST in control makes 114.4±5.17 U/L, and significant change of level of enzyme is observed only in the second experimental group – 163.98±9.88 U/L. (Figure 3)

The LDG level in control made 941.0±59.6 U/L, in the first experimental group – 1025.6±88.6 U/L, and in the second – 1314.0±78.81 U/L.

Respectively, ACT/ALT relation in control makes 2.28±0.12, in the first experimental group decreases a little, making 2.08±0.14, and in the second makes 0.95±0.1.

Results of the histologic analysis showed that in a liver of rats of the first experimental group developed steatopepatitis, characterized by focal sites of a necrosis with lymphomacrophagal elements, and also fatty dystrophy of cells of a liver. Numerous sites of a liver with the mixed type of a steatosis, single necrotizing hepatocytes and the centers of a micronecrosis (Figure4) are noted.
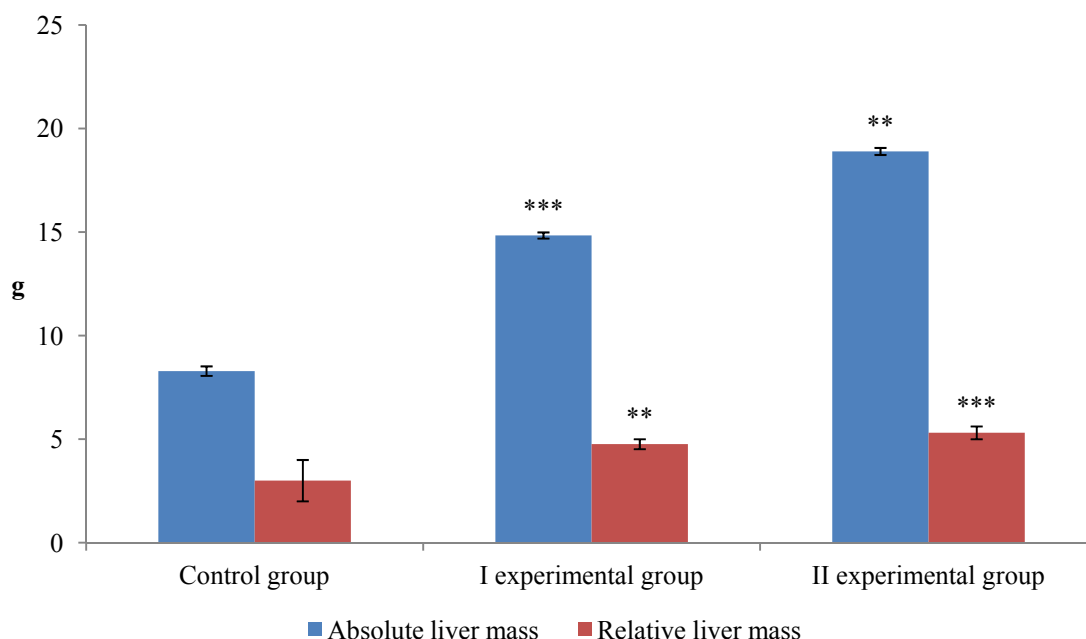

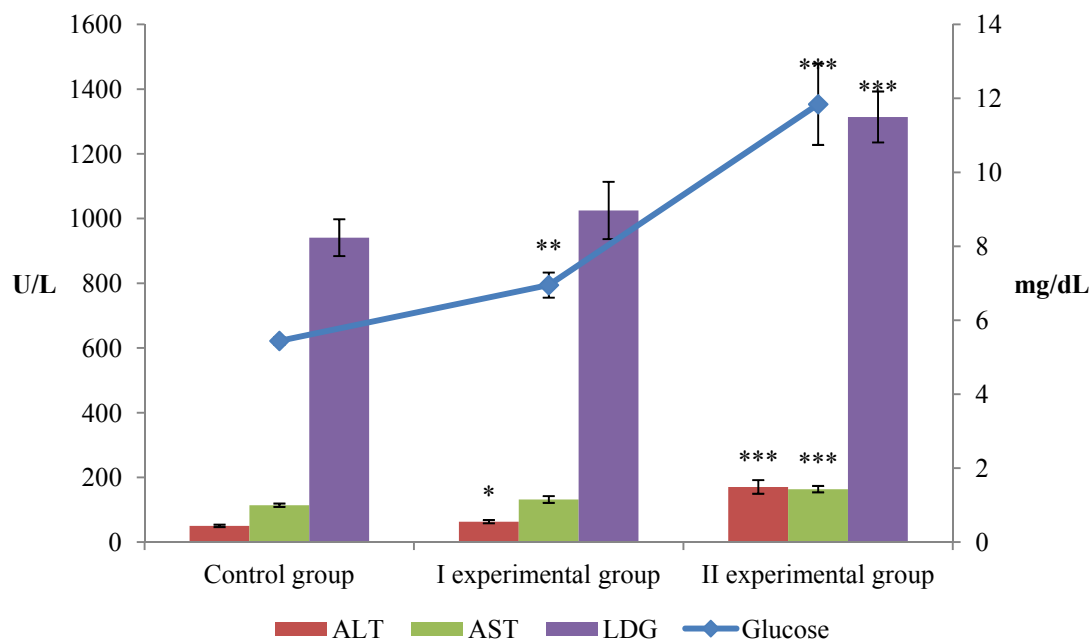Figure 2. Weight of absolute and relative liver mass of animals of controlandexperimental groups

Figure 3. Level of glucose ,ALT,AST and LDG in serum of blood of animals of controlandexperimental groups

Histologic structure of a liver of rats of the second experimental group underwent the considerable changes expressed in increase of the area of the necrotizing sites (14-17% of total area of the studied sites of body) surrounded with lymphomacrophagal infiltrate, violation of a frame structure of segments of a liver. The trabecularity of a liver isn't expressed, hepatocytes settled down randomly, sinusoids weren't traced. Expansion of large portal paths due to growth of a stroma and filling with its inflammatory infiltrate was noted. Some portal paths were with necrotic changes of vessels. Walls of such vessels were poorly differed and in certain cases were broken. The destruction of biliary channels accompanied with the big centers of inflammatory infiltrate was also observed (Figure 5).
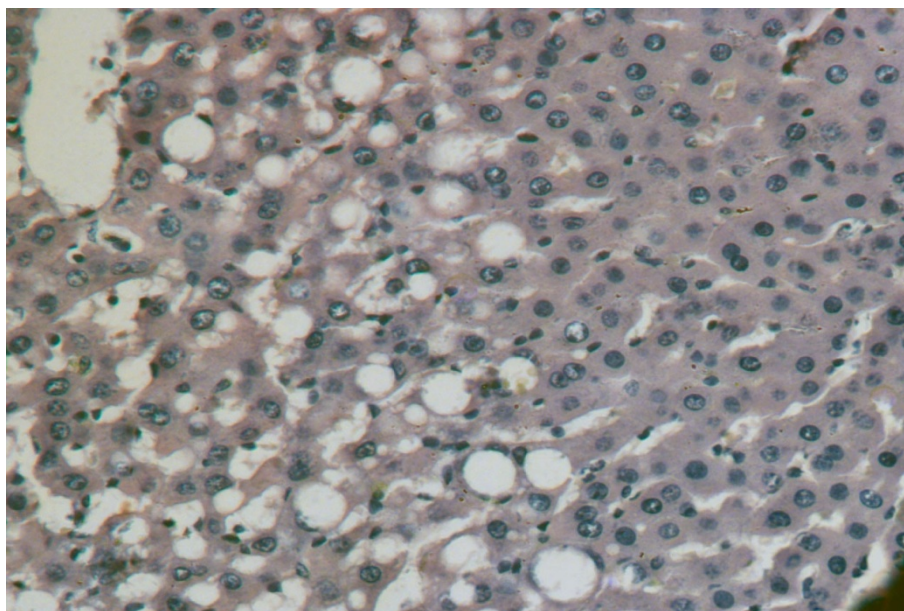

Figure 4. Liver of rat of I experimental group.H&E, ×200

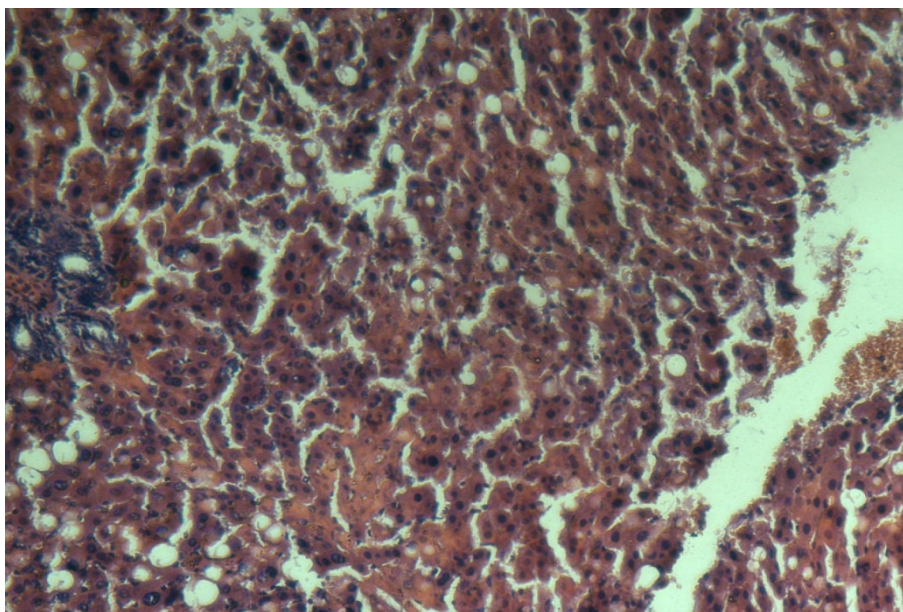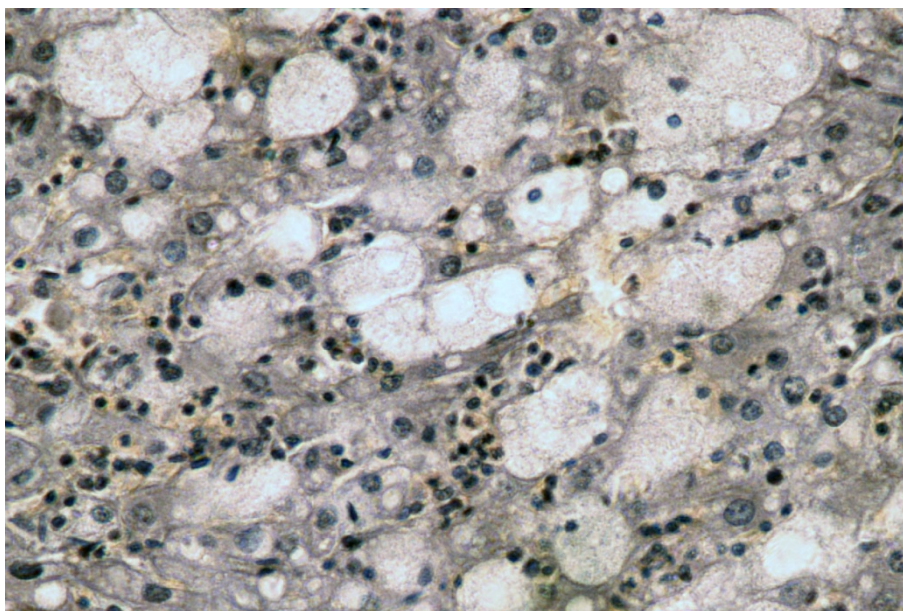Figure 5. Liver of rat of II experimental group. H&E, ×100

Figure 6. Liver of rat of II experimental group.H&E, ×200

On the preparations with van Gieson'spirofuchsin stain in a preportal zone were accurately seen collagenic fibers that is the proof of the matured liver fibrosis.

4. Discussion

Thus, it was succeeded to receive the model of non-alcoholic steatohepatitis which is characterized by obesity, violation of the structural organization, a tissue necrosis, formation of fibrosis of a liver. The developed model of non-alcoholic steatohepatitis allows to study also staging of development of a fatty desease of the liver which is characterized by formation of a steatohepatitis and fibrosis. Results of research testify to undoubted appeal of use of this model. The offered NASH model can be used in the field of chronic experiments for an assessment of extent of violation of a functional condition of hepatobiliary system and search of effective

pharmacotherapeutichepatoprotective agents.

Besides, the offered model is carried out in the absence of mortality of animals. Also is important that it is used the most available species - rats, that meets the research objectives. Creation of such model does possible carrying out experiments which statement in clinical practice is impracticable. The offered NASH model can be used for carrying out search and test of action of the hepatoprotective preparations.

5. Conclusion

Thus, the conducted researches allowed to receive new experimental model of non-alcoholic steatohepatitis, which cornerstone is the complex combination of the pathological factors initiating the cascade of the damages of a liver leading to development of the resistant morphofunctional violations of organ. The offered model is simple in performance and is characterized by a high degree of reproducibility and lack of a lethal outcome among experimental animals.

The offered way of modeling allows coming nearer in a bigger measure to the clinical course of pathological process in a liver.

The offered way of modeling reproduce the reliable model of a steatohepatitis, confirmed by the morphological analysis of a liver, histochemical and biochemical indicators . Besides, this way provides possibility of studying of development of a steatohepatitis in dynamics.

Acknowledgments

Conflict Of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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