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Serum level of laminin in rats fed with a high-fat diet with sulodexide administration

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ABSTRACT

Background. Increased consumption of animal fat with food contributes to the accumulation of lipids both in the blood and in individual cell structures. Excess fat initiates oxidative stress reactions, which may result in a violation of the structural and functional integrity of cells, in particular, hepatocytes and endotheliocytes. Cytolysis may release specific liver enzymes and activate synthesis of extracellular matrix components, one of the markers of which is a non-collagen glycoprotein laminin.

The drug sulodexide, having a pronounced angioprotective, hypolipidemic, and fibrinolytic effects, contributes to restoration of a number of metabolic disorders.

Aim. To study the content of lipid metabolism parameters, major enzymes of hepatic cytolysis, and laminin in the blood of rats fed with a high-fat diet against the background of sulodexide administration.

Materials and methods. For the study, outbred rats were selected, which were divided into three groups – two experimental groups and one control group. The rats of the first and second experimental groups were fed with a diet with a high content of animal fat (44% of the daily calorie content) for 35 days. In addition, the rats of the second experimental group were daily subcutaneously injected with sulodexide at a dose of 8.5 LRU/kg in terms of the animal's body weight for 35 days. Starting from day 36 of the experiment, the rats of the control group, as well as the rats of the two experimental groups were fed with a standard diet of the vivarium. The animals were decapitated and blood was taken on day 21, 35, and 60 of the experiment. In the blood serum, the levels of the main lipid metabolism parameters, specific liver enzymes, and laminin were determined.

Results. An increase in the body weight of animals and the level of the studied lipid metabolism parameters in the blood serum was revealed. It is likely that the structural integrity of hepatocytes was affected with the release of liver enzymes into the bloodstream and an increase in their content in the blood of rats. In addition, synthesis of extracellular matrix components was activated with an increase in the serum level of laminin, which performs important structural and regulatory functions.

Conclusion. The use of sulodexide had a favorable effect on the studied metabolic disorders caused by a high-fat diet. It resulted in the normalization of the synthesis of laminin, one of the major non-collagen proteins of the extracellular matrix.

Keywords: high-fat diet, lipid metabolism, liver enzymes, laminin

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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Izhevsk State Medical Academy (Protocol No. 652 of 23.04.2019).

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Содержание ламинина в сыворотке крови крыс в условиях высокожировой диеты при коррекции сулодексидом

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РЕЗЮМЕ

Повышенное поступление животного жира с пищей способствует накоплению липидов как в крови, так и в отдельных клеточных структурах. Излишек жиров инициирует реакции окислительного стресса, следствием которых может явиться нарушение структурной и функциональной целостности клеток, в частности гепатоцитов и эндотелиоцитов. В результате цитолитических процессов возможно высвобождение специфических ферментов печени и активация синтеза компонентов межклеточного матрикса, одним из маркеров которого является неколлагеновый гликопротеин ламинин.

Препарат «Сулодексид», обладая выраженным ангиопротекторным, гиполипидемическим, фибринолитическим действием, участвует в восстановлении ряда обменных нарушений.

Цель. Изучение содержания показателей липидного обмена, основных ферментов цитолиза гепатоцитов и ламинина в крови крыс при высокожировой диете на фоне коррекции сулодексидом.

Материалы и методы. Для исследования были отобраны беспородные крысы, которых разделили на три группы – две опытных и одну контрольную. Крысам первой и второй опытных групп была назначена диета с повышенным содержанием животного жира (44% от суточной калорийности) в течение 35 сут. Крысам второй опытной группы ежедневно в течение 35 сут подкожно вводился сулодексид в дозировке 8,5 ЛЕ/кг в перерасчете на массу тела животного. Крысы всех групп с 36-х сут опыта находились на стандартном рационе вивария. Декапитацию животных и забор крови проводили на 21, 35 и 60-е сут опыта. В сыворотке крови определяли содержание основных показателей липидного обмена, специфических ферментов печени и ламинина.

Результаты. Наблюдался рост массы тела животных, увеличение содержания изучаемых показателей липидного обмена в сыворотке крови. Вероятно, затрагивалась структурная целостность гепатоцитов с вымыванием печеньспецифических ферментов в кровяное русло и повышением их содержания в крови крыс. Активировался синтез компонентов внеклеточного матрикса с возрастанием содержания в крови исследуемого неколлагенового белка – ламинина, выполняющего значительную структурную и регуляторную функцию в его организации.

Заключение. Применение сулодексида благоприятно повлияло на исследуемые метаболические нарушения, вызванные приемом пищи с повышенным содержанием жира, в том числе привело к нормализации синтеза одного из основных неколлагеновых белков межклеточного матрикса – ламинина.

Ключевые слова: высокожировая диета, липидный обмен, ферменты печени, ламинин

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Obesity and metabolic syndrome have reached epidemic proportions in the 21st century. The urgency of the problem of weight gain is associated with an increased risk of development and progression of atherogenic dyslipidemia, cardiovascular diseases, and nonalcoholic fatty liver disease (NAFLD) [1]. NAFLD is characterized by low-grade inflammation in hepatocytes, which ultimately can lead to activation of connective tissue growth and formation of liver fibrosis [1].

A number of previous studies have shown a correlation between the accumulation of fat in hepatocytes and the stage of liver fibrosis [2]. Increased consumption of fat contributes to an increase in the supply of free fatty acids (FFA) to liver cells, a decrease in the rate of beta-oxidation of FFA, as well as an increase in the synthesis or secretion of very-low-density lipoproteins (VLDL) in the liver, and then low-density lipoproteins, formed from VLDL following hydrolysis of triglycerides under the action of lipoprotein lipase located on the capillary endothelium.

A significant contribution to fibrogenesis is made by oxidative stress following lipid peroxidation with the formation of highly reactive toxic compounds and activation of inflammatory reactions with the release of proinflammatory cytokines. The emerging oxidative stress and excess of triglycerides in hepatocytes can cause their cytolysis with the release of liver enzymes and, as a consequence, active synthesis of the extracellular matrix. High blood level of triglycerides in the composition of low-density lipoproteins can have a damaging effect on endothelial cells. Laminin, a non-collagen protein of connective tissue, can be one of the markers of these processes.

Laminins are a family of large glycoproteins that bind to one other and form about 15 heterotrimeric macromolecules. The molecular weight of laminin ranges from 400 to 900 kDa. Currently, 16 laminin isoforms have been identified in mammals [3]. Laminins are some of the components of the basement membranes in various tissues, mediating their interaction with cells. They contribute to the formation of an integral structure between cellular receptors and the basement membrane due to their polymerization and act as key molecules in the formation of unique tissue structures. Together with other non-protein components of the extracellular matrix, they participate in cell adhesion, tissue reconstruction, and maturation of collagen fibers.

The drug sulodexide (Vessel Due F®) is an anticoagulant that has antiplatelet, antithrombotic, angioprotective, hypolipidemic, and fibrinolytic effects. The active ingredient is an extract from the mucous membrane of the small intestine of animals; it is a natural mixture of a low-molecular-weight heparin (80%) and dermatan sulfate (20%) [4]. The mechanism of the angioprotective effect is associated with restoration of the structural and functional integrity of vascular endothelial cells. It normalizes blood rheology by lowering the level of triglycerides and reducing blood viscosity [4].

The aim of the research was to study the content of lipid metabolism parameters, major enzymes of hepatic cytolysis, and laminin in the blood of rats fed with a high-fat diet against the background of sulodexide administration.

MATERIALS AND METHODS

The study was carried out on white outbred male rats with an initial body weight of 200–250 g. Work with rodents was carried out in accordance with the Order of the Ministry of Health of the Russian Federation No. 199n of 01.04.2016 “On the approval of the rules of good laboratory practice”. The study was approved by the local Ethics Committee at Izhevsk State Medical Academy (Protocol No. 652 of 23.04.2019).

The animals were kept in cages at 23 ± 2°C with 12h / 12h light – dark regime and had free access to food and water. All animals were divided into three groups – a control group (15 rats) and two experimental groups (15 animals each). The rats of the control group were fed with a standard vivarium diet. For 35 days, the animals of the two experimental groups received a high-fat diet – 44% of lard and 9% of vegetable oil from the daily diet [5]. In addition, for 35 days, the rats in the second experimental group received daily subcutaneous injections with Vessel Due F® at a dose of 8.5 LRU / kg in terms of the animal’s body weight [6]. From day 36 to day 60 of the experiment, all the rats were fed with a standard diet of the vivarium. On day 60 of the experiment, changes in the studied parameters were assessed in the long run [7].

The body weight of the animals was determined before the start of the experiment and at each time interval studied. Fasted animals were removed from the experiment on days 21, 35, and 60 by decapitation under short-term ether anesthesia. The material for the research was blood taken from the cervical vein. Blood serum was obtained from blood samples by centrifugation (3,000 rpm for 15 minutes) to determine biochemical parameters and laminin. An

automatic chemistry analyzer AU-480 (Beckman Coulter, USA) with appropriate test systems was used to determine the concentration of the main lipid metabolism parameters (total cholesterol (TC), high-density lipoproteins (HDL) and low-density lipoproteins (LDL), triglycerides (TG)), as well as the activity of the major liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). The laminin concentration was determined by the enzyme-linked immunosorbent assay (ELISA) using the Laminin ELISA Kit (USA).

Statistical processing of the results was carried out using the SPSS Statistics software. Descriptive statistics are presented as the median and the interquartile range *Me* [Q_1; Q_3]. The data obtained did not follow a normal distribution; therefore, the statistical significance of the differences was calculated using the nonparametric Mann – Whitney test. The value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The body weight of the animals from the experimental group 1 fed with a high-fat diet increased by day 35 of the experiment and continued to increase till the end of the experiment. The body weight of the animals from the experimental group 2 who received a high-fat diet and injections of sulodexide did not differ significantly from the control values (Table 1).

After consumption of high-fat food, the following changes in the lipid profile were observed in the blood serum of the rats in the experimental group 1: an increase in the concentration of TC by 165.3%, LDL – by 458.8%, TG – by 522.5%, and HDL – by 58.6% compared with the control by day 60 of the experiment (Table 2).

Table 1

Change in body weight of the experimental rats compared with the control, *n* = 5

Conditions of the experiment	Experiment time, days		
	Day 21	Day 35	Day 60
Diet	15.2; $p = 0.089$	25.9; $p = 0.041$	17.6; $p = 0.039$
Correction	0.1; $p = 0.405$	7.4; $p = 0.076$	15.2; $p = 0.118$

Table 2

Biochemical parameters in the blood serum of rats fed with a high-fat diet, mmol / l, *Me* [Q_1; Q_3]

Parameter	Experiment time, days					
	Day 21		Day 35		Day 60	
	Control, *n* = 5	Experiment, *n* = 5	Control, *n* = 5	Experiment, *n* = 5	Control, *n* = 5	Experiment, *n* = 5
TC	0.78 [0.75; 0.92]	1.69 [1.69; 1.81] $p = 0.009$	0.85 [0.81; 0.91]	1.98 [1.89; 2.01] $p = 0.007$	0.89 [0.84; 0.96]	2.07 [1.85; 2.11] $p = 0.009$
HDL	0.46 [0.41; 0.47]	0.74 [0.73; 0.79] $p = 0.012$	0.52 [0.50; 0.59]	0.69 [0.67; 0.7] $p = 0.025$	0.55 [0.51; 0.59]	0.73 [0.71; 0.79] $p = 0.008$
LDL	0.17 [0.17; 0.23]	0.46 [0.45; 0.49] $p = 0.009$	0.19 [0.17; 0.22]	0.56 [0.47; 0.57] $p = 0.009$	0.21 [0.14; 0.33]	0.95 [0.56; 1.2] $p = 0.009$
TG	0.40 [0.4; 0.47]	1.77 [1.48; 2.89] $p = 0.009$	0.46 [0.41; 0.57]	1.90 [1.87; 1.91] $p = 0.011$	0.52 [0.49; 0.56]	2.49 [1.58; 2.7] $p = 0.016$

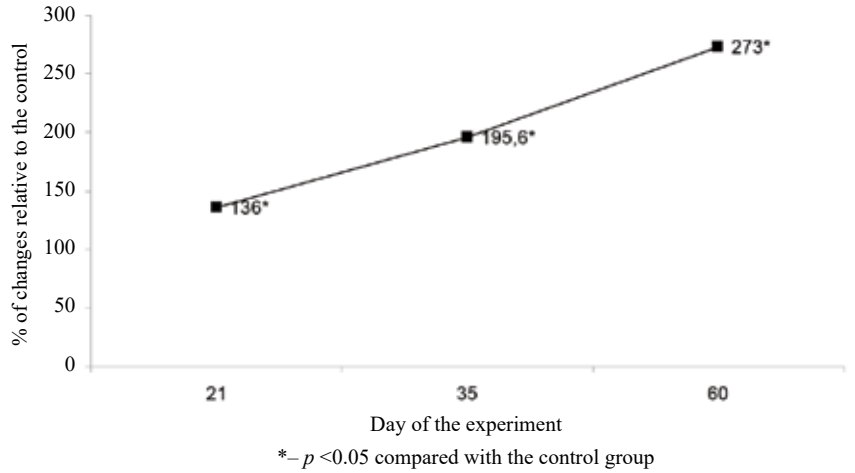
It is reasonable to expect these changes from the standpoint of assimilation of exogenous lipids due to their increased absorption and enhanced synthesis of VLDL in the liver, and then LDL in the vascular endothelium. Excess of TG is deposited in the form of fatty vacuoles in hepatocytes, which leads to the formation of fatty liver disease – steatosis [8]. As a result, lipid peroxidation processes are activated, proinflammatory cytokines are synthesized, and oxidative stress develops.

These processes may result in hepatic cytolysis, leading to the release of intracellular enzymes, such as ALT, AST, ALP, and LDH, and their accumulation in the blood. In our experiment, a statistically significant increase in ALT activity by 104.4%, 184.1%, 219.3% ($p < 0.05$) on day 21, 35, and 60 of the experiment, respectively, and a tendency toward elevation of AST, ALP, and LDH with a statistically significant increase by 27.8%, 204.7%, and 42.6%, respectively, by day 60 of the experiment were noted.

In the case of persistent damage, slowdown in regeneration and replacement of hepatocytes with an excess amount of extracellular matrix proteins were observed [2]. In addition, the excess of TG and TC following free radical oxidation might have a destructive effect on the vascular endothelium. Laminin, the basement membrane glycoprotein, may be one of the markers of ongoing pathological changes.

Laminin is a structural non-collagen glycoprotein of the basement membrane consisting of three short arms with globules and one long arm [9]. Each laminin chain consists of several domains on which active centers of interaction with various biologically active substances, such as type IV collagen, fibronectin, and nidogen, are located. Nidogen occupies a prominent place in the structure of the cellular matrix having a covalent bond to collagen and forming an insoluble, non-covalently bound complex with laminin. This complex is fixed with cells, which determines the main function of laminin as an adhesive protein of various epithelial and mesenchymal cells providing tissue resistance to stretching and affects cellular growth, morphology, differentiation, and mobility [10]. Therefore, when the cells are exposed to a damaging factor, the release of extracellular matrix components into the bloodstream is likely.

In the course of the experiment, an increase in the laminin content was observed already on day 21 of the experiment and by the end of the experiment (Fig. 1).



*– p <0.05 compared with the control group

Fig. 1. Changes in the concentration of laminin against the background of a high-fat diet relative to the control, %

The study of biochemical liver parameters against the background of sulodexide administration and a high-fat diet showed that the activity of the major enzymes of hepatic cytolysis decreased at the indicated points of the experiment, with the highest value on day 60 compared with similar parameters in the first group of animals (Fig. 2).

The hepatoprotective effect of the drug may also be associated with its hypolipidemic effect, an increase in the activity of lipoprotein lipase, and, therefore, with increased breakdown of TG in the composition of lipoproteins, and, as a consequence, with a decrease in the concentration of products of their peroxidation and restoration of hepatocytes. According to the results of the experiment, after administration of sulodexide, a tendency toward a decrease in the concentration of the studied lipid profile parameters was noted: TC decreased by 26%, HDL – by 1.4%, LDL – by 52%, and TG – by 26% on day 21 of the experiment. An even greater decrease was recorded on day 35 of the experiment: TC reduced by 37%, HDL – by 30%, LDL – by 18%, and TG – by 64% ($p < 0.05$) with a pronounced decrease by day 60 of the experiment (Fig. 3).

A decrease in the difference in these parameters against the background of sulodexide administration compared with the control values by the end of the experiment is worth noting (Fig. 4). The angioprotective effect of sulodexide associated with restoration of the structural and functional integrity of the vascular endothelium, normalization of negative electric charge density, and decreased basement membrane thickness and extracellular matrix production explains a decrease in the laminin concentration observed against the background of the diet and after administration of the drug by 26.3%, 22.6%, and 37.4% on day 21, 35, and 60 of the experiment, respectively (Fig. 5).

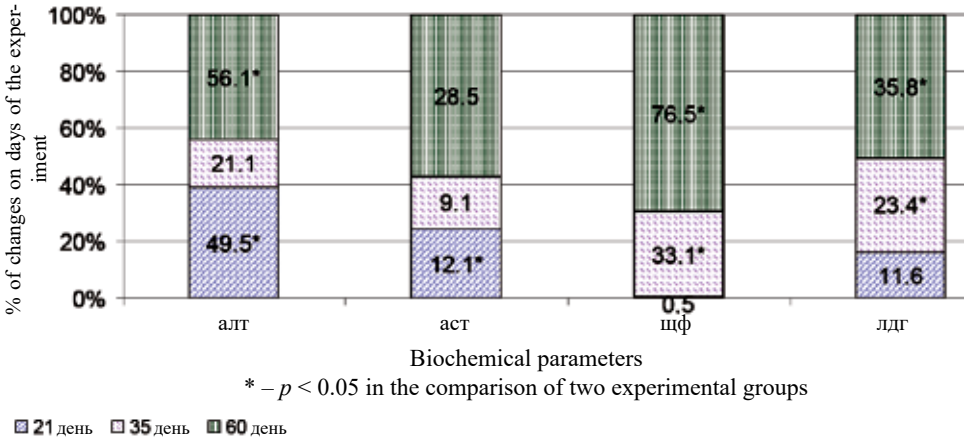


Fig. 2. Changes in the activity of enzymes involved in hepatic cytolysis under the conditions of a high-fat diet and sulodexide injections by days of the experiment, %

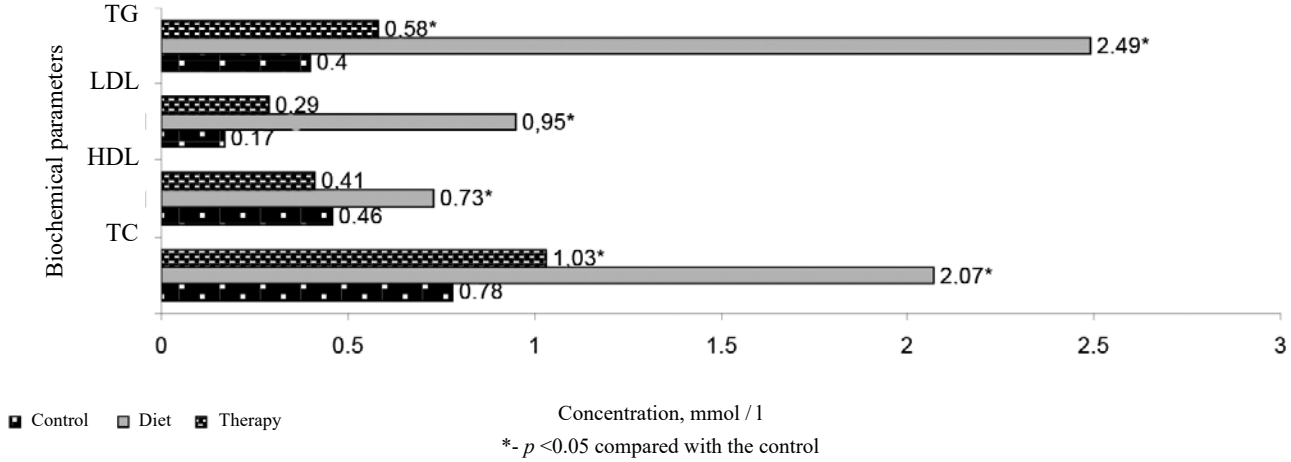


Fig. 3. Parameters of lipid metabolism under conditions of a high-fat diet and against the background of sulodexide administration by the end of the experiment

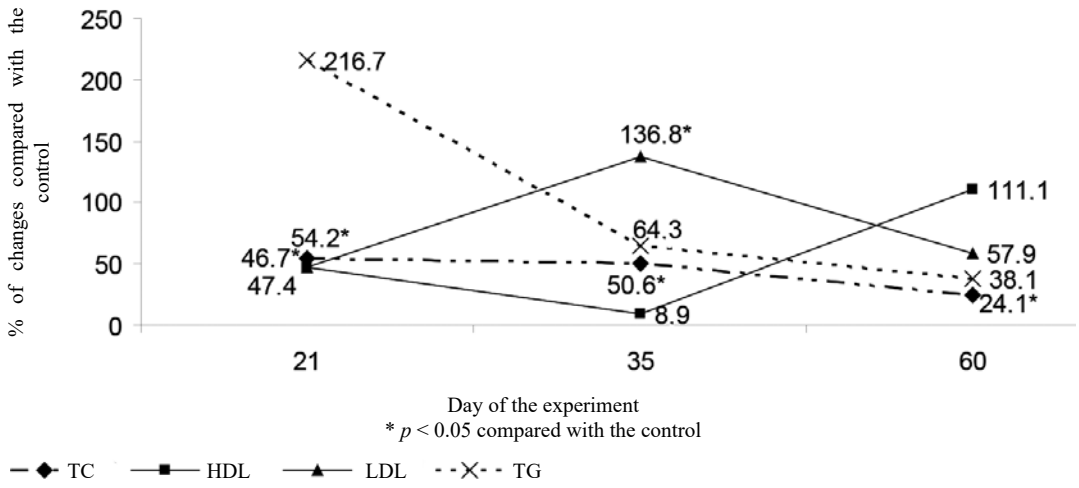


Fig. 4. Dynamics of the lipid profile parameters against the background of sulodexide administration relative to the control

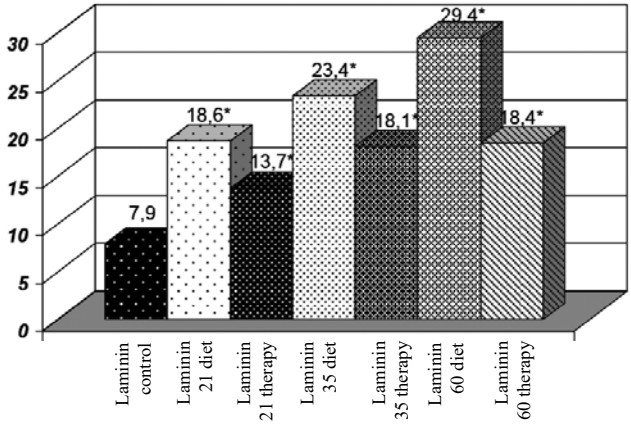


Fig. 5. Concentrations of laminin against the background of the diet and sulodexide administration: the abscissa is the time of the experiment, days; the axis of ordinates is the laminin concentration in the blood serum, ng / ml. * – $p < 0.05$ compared with the control. Due to a lack of statistically significant differences in the control values, the mean value for the control was calculated for all days of the experiment

The analysis of the data obtained allows to conclude that the administration of sulodexide may be reasonable for managing steatosis and liver fibrosis and restoring the integrity of the vascular endothelium, which is typical of a high-fat diet.

CONCLUSION

The results of the experiment indicate that a high-fat diet leads to a change in the concentration of lipid metabolism parameters in the blood serum of rats. This can initiate destructive processes in the liver cell membrane with the release of the main biochemical markers of hepatic cytolysis (ALT, AST, ALP, LDH) into the bloodstream, as well as endothelial dysfunction with an increase in the laminin level in the blood of animals.

The studied drug sulodexide has a rather pronounced hypolipidemic and angioprotective effect. It is able to reduce the degree of hepatic cytolysis due to the activation of lipolysis and is likely to restore the structure of the basement membrane in the vascular endothelium. This is one of the reasons for applying this drug in clinical practice.

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