ISSN 2509-4327 (print)
ISSN 2510-4780 (online)

Deutscher Wissenschaftsherold
German Science Herald

№ 4/2017

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Impressum
Deutscher Wissenschaftsherold – German Science Herald
Wissenschaftliche Zeitschrift
Herausgeber:
InterGING
Sonnenbrink 20
31789 Hameln, Germany
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Email: info@dwherold.de
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Auflage: № 4/2017 (September) – 30
Redaktionsschluss September, 2017
Erscheint vierteljährlich
Editorial office: InterGING
Sonnenbrink 20
31789 Hameln, Germany
Tel.: + 49 51519191533
Fax.:+ 49 5151 919 2560
Email: info@dwherold.de
Deutscher Wissenschaftsherold - German Science Herald is an international, German/English language, peer-reviewed, quarterly published journal.
№ 4/2017
Passed in press in September 2017
Druck: WIRmachenDRUCK GmbH
Mühlbachstr. 7
71522 Backnang
Deutschland

INDEXING: Google Scolar, WorldCat, Index Copernicus, InfoBase Index, Journal Index, Citefactor, International Scientific Indexing, JIFACTOR, Scientific Indexing Services, International Institute of Organized Research.

REDAKTIONSKOLLEGIUM / INTERNATIONAL EDITORIAL BOARD:

Lists of references are given according to the Vancuver style

DDC-UDC 616.36-003.826:615.036.8 DOI:10.19221/2017424

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CORRECTION OF METABOLIC DISORDERS CAUSED BY NON-ALCOHOLIC FATTY LIVER DISEASE

Abstract. *We have developed an effective way to correct metabolic disorders caused non-alcoholic fatty liver disease, by selecting medical remedies, considering peculiarities of the pathogenetic mechanisms of the development of metabolic disorders in this disease. In order to correct metabolic disorders in non-alcoholic fatty liver disease, a biologically active compound of (S) -2.6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate was used in the experiment with rats with steatosis in a dose of 50 mg / Kg (solved with Ringer-Locke solution to 25 ml / kg) intraperitoneally once daily for 30 days. The study established that a biologically active compound of (S) -2.6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate in a dose of 50 mg / kg has a high hepatoprotective and anti-hepatotoxic activity, which provides useful properties in the treatment of non-alcoholic fatty liver disease.*
Key words: *liver diseases, steatosis, treatment*

Introduction. In non-alcoholic fatty liver disease (NAFLD), an excessive mobilization of free fatty acids from peripheral depots of lipids and their ingress into hepatocytes develops. This phenomenon is caused by a tissue decreased sensitivity to insulin and disorders in glucose supply to cells, which ultimately leads to an increase in lipolysis rate in the adipose tissue with an increase in the concentration of free fatty acids in the blood (Randl biochemical cycle) [1]. Free fatty acids, in turn, disturb the endothelial function due to the production of free radicals, activation of protein kinase C, and increased dyslipidemia. In this regard, the liver can be considered both as the target organ and as a direct source of proinflammatory cytokines, which determine the cascade of inflammatory reactions, which lead to a damage to the smooth muscle cells, endothelial dysfunction and a damage to the hepatocytes themselves, thus forming a "vicious circle" [2].

To date, there is no doubt that liver steatosis and non-alcoholic steatohepatitis are severe stages of NAFLD associated with metabolic disorders. There are reports that the liver inflammation is induced by its steatosis. Endoplasmic reticulum and oxidative stress are responsible for this process [8]. A stress of the endoplasmic reticulum in the liver and adipose tissue was simulated in mice with genetically determined and induced by a diet forms of obesity. It turned out that this process leads to a disorder in the insulin signaling pathways. Hepatocytes constitute about 2/3 of the total number of liver cells, and other cellular elements are represented by biliary epithelial cells, sinusoidal endothelial cells, Kupffer cells, star cells, dendritic cells, and by lymphocytes.

Recent studies have shown the role of hyperhomocysteinemia in the development and progression of NAFLD [5, 7]. Due to the multifactorial and heterogeneous nature of non-alcoholic fatty liver disease, the therapeutic approaches should be comprehensive as well.

A known way to prevent the progression of the pathological process in the liver of patients with NAFLD is to administer antioxidants to them-ascorbic acid and vitamin E (tocopheryl acetate) in medium therapeutic doses [4]. With the use of this method, improvement of lipoperoxidation indexes and reduction of the terms of further progression of the pathological process in the liver were noted, however, in 15-20% of cases, the pathological process in the liver parenchyma of

patients with NAFLD progresses, which contributes to the formation of fibrosis or even cirrhosis of this organ.

In the treatment of NAFLD differentiated use of drugs that have a pronounced anti-oxidant, anti-inflammatory, membrane-protective, immunomodulatory properties is pathogenetically justified and they have a positive therapeutic effect on many links of the disease pathogenesis. Today an original drug was created by synthesizing the active substance of (S) -2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (its working title is Angiolin), which combines fragments of molecules of Thiotriazoline and L-lysine aescinat in its structure and has high antiischemic, cardioprotective, neuroprotective, antioxidant and anti-inflammatory properties [6]. Intravenous administration of angiolin to animals with myocardial ischemia led to normalization of the ratio of thiol-disulfide system and nitric oxide system in the myocardium, as well as an increased activity of endothelial NO synthase, decreased nitrotyrosine levels, increased levels of reduced glutathione and cysteine, and an increased glutathione reductase activity [3]. Applying biologically active compound Angiolin in the comprehensive treatment of NAFLD is rational in terms of its effect on metabolic processes occurring in the liver, and to correct the endothelial dysfunction as well as to prevent cardiovascular complications.

Objective. To determine the effectiveness of the correction of metabolic disorders in NAFLD by means of biologically active compound Angiolin.

Material and methods. Experimental studies involved 50 white non-linear male rats weighing 180-200 g. Before the experiments, the animals had been kept in quarantine for 10 days. During this period, animals had received an adequate standard semisynthetic starch-casein diet. Subsequently, the animals were divided into 2 groups: the control one included10 intact animals, which continued to be on the same diet under the conditions similar to those of the experimental group, and the experimental group, consisting of 40 rats, in which a model of liver steatosis was created by keeping them for 8 weeks on a hypercaloric diet high in fat and in cholesterol containing about 30% fat (mostly saturated lipids) with cholesterol (obtained by mixing 2 g of cholesterol and 10 g of pork fat from 88 g of normal balanced diet granules) [Kucera O. Experimental models of non-alcoholic fatty liver disease in rats / O. Kucera, Z. Cervinkova // World J. Gastroenterol. - 2014. – Vol. 20, № 26. – P. 8364-8376]. After creating the liver steatosis model for the animals, they continued to be kept on a high-fat diet, but part of the animals of the experimental group (20 rats) were administered additional 4 weeks of biologically active compound Angiolin in a dose of 50 mg per kg of their body weight (solved with Ringer-Locke solution to 25 ml / kg) intraperitoneally , and a part of the animals (20 rats) were only injected the Ringer-Locke solution (25 ml / kg) intraperitoneally for 30 days.

On the 30th day after the NAFLD model was developed, all animals were taken serum samples under thiopental anesthesia (40 mg / kg) for biochemical studies and samples of the liver tissue for morphological examination. After the centrifugation the blood serum was checked according to unified conventional methods for: the activity of enzyme markers of cytolysis (alanine aminotransferase, aspartate aminotransferase) and cholestasis (alkaline phosphatase, the level of total bilirubin and its fractions), the protein synthesis of the liver (total protein, albumins).

Results and discussion. At present, the properties of (S) -2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5-thioacetate to correct metabolic disturbances with NAFLD have not been described in the scientific literature, and no mention was made of the medical use of the substance in the NAFLD treatment scheme.

Using biologically active compound (S) -2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5- thioacetate 50 mg / kg (solved with solution Ringer-Locke 25 ml / kg) once a day for 30 days can reduce cytolysis syndrome (reduce such biochemical parameters as the activity of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase) to reduce cholestasis syndrome (decrease in alkaline phosphatase), leads to normalization of protein synthesis liver function (levels of total protein, albumins, albumin / globulin ratio) to improve the morphological status of hepatocytes, indicating

normalization of structural and functional state of the liver and thus leads to the achievement of the technical effect, that increases the effectiveness of therapy.

The results of the experiment showed that the test compound has hepatoprotective effect. Studying the intensity of the cytolysis syndrome in rats while correcting the experimental model of NAFLD showed that the activity of the alanine aminotransferase was lower in the use of angiolin (by 17.3%, p <0.05) compared to untreated animals with the NAFLD model. The gamma-glutamyltranspeptidase activity was also lower compared to untreated animals when the compound was used (table 1), namely: by 32.4% at the time of administration of Angiolin (p <0.05). The activity of aspartate aminotransferase did not approach the control values when using the compound, however, this index was higher by 21.3% and statistically differed by 8.7% (p <0.05) from that in untreated animals after the administration of angiolin.

Since the general indirect bilirubin rate and alkaline phosphatase activity are the biochemical markers of cholestasis syndrome, we used these rates to assess the effectiveness of Angiolin (table 2). The indicator that most clearly presented the cholestasis syndrome in rats with modeled NAFLD was the activity of alkaline phosphatase, which was by 31.6% higher (p <0.05) in untreated animals compared to the intact group. When Angiolin was administered the activity of alkaline phosphatase was lower (by 27.6%, p <0.05) compared with the untreated group. Studying the content of general and indirect bilirubin, we did not establish statistically significant differences with the use of Angiolin (table 2).

The total protein rate was most closely related to that in animals of the intact group with administered Angiolin (p <0.05). A similar trend was observed in determining the albumin content. Albumin content was higher with administered Angiolin (by 30.9%, p <0.05) compared to non-treated NAFLD models and close to that in intact animals. When using Angiolin, a larger albumin / globulin ratio was recorded, the difference in the group of untreated animals using angiolin was 31.3% (p <0.05) (table 3).

Table 1

Features of the biochemical parameters of the cytolysis syndrome in rats with modeled NAFLD and after its correction by angiolin of ((S) -2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5-thioacetate) (M ± m)

	Intact rats (n=10)	Rats with modeled NAFLD	
		No treatment (n=10)	Correction with Angiolin (n=10)
Aspartate aminotransferase, (un/l)	160,26±14,72	223,08±6,53*	203,74±5,37*#
Alanine aminotransferase (un/l)	182,43±12,38	241,62±17,72*	199,85±7,35#
gamma-glutamyltranspeptidase (un/l)	9,86±0,32	15,62±0,86*	10,56±0,44#

*Note: * - statistically significant difference with indices in intact animals (p<0,05); # - statistically significant difference with indices in animals with modeled NAFLD without treatment* (p<0,05);

Table 2

Features of biochemical parameters of cholestasis syndrome in rats with modeled NAFLD and after its correction by angiolin of ((S) -2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5-thioacetate) (M ± m)

	Intact rats (n=10)	Rats with modeled NAFLD	
		No treatment (n=10)	Correction with Angiolin (n=10)
Alkaline phosphatase (un/l)	381,16±24,14	557,33±23,24*	403,45±23,24#
Total bilirubin (µmol / L)	8,63±0,51	9,83±0,62	8,68±0,47
Indirect bilirubin (µmol / L)	4,74±0,52	4,84±0,54	4,75±0,49

*Note: * - statistically significant difference with indices in intact animals (p<0,05); # - statistically significant difference with indices in animals with modeled NAFLD without treatment (p<0,05);*

Table 3

Features of protein synthesis liver function in rats with modeled NAFLD and after its correction by angiolin of ((S) -2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5-thioacetate) (M ± m)

	Iintact rats (n=10)	Rats with modeled NAFLD	
		No tratment (n=10)	Correction with Angiolin (n=10)
Total protein(g/l)	82,45±1,15	67,14±2,07*	79,18±2,13#
albumins (g/l)	49,18±2,36	31,16±2,68*	45,12±1,54#
albumins/globulins ratio	1,61±0,14	0,90±0,08*	1,31±0,22#

*Note: * - statistically significant difference with indices in intact animals (p<0,05); # - statistically significant difference with indices in animals with modeled NAFLD without treatment (p<0,05);*

The results of the morphological study of liver tissue in experimental animals with modeled NAFLD against the background of the introduction of biologically active compound of (S) -2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5-thioacetate 50 mg / kg show the recovery of lobular structure of the liver parenchyma compared to animals with a modeled NAFLD without correction. As an evidence of recovery processes activation in the liver during treatment with biologically active compound of (S) -2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5-thioacetate was a significant intensification of dual hepatocytes mass index, which was reliably higher than in animals with modeled NAFLD without correction, reliably more functional nuclear cell index than in the animals with modeled NAFLD without correction. Thus, the effectiveness of the correction method was proved by the experimental research.

Conclusion. This study found that a biologically active compound of (S) -2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate in a dose of 50 mg / kg has a high hepatoprotective and anti-hepatotoxic activity which provides useful properties in the treatment of NAFLD. The new properties of Angiolin allow performing an effective therapy of experimental liver damage - NAFLD, and the invention can be used in medicine.

Prospects of further research. It would be perspective in the future to investigate how Angiolin affects the function of endothelial cells of the liver sinusoids and releasing biologically active substances produced by the endothelium.

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CONTENT:

Deutscher Wissenschaftsherold
German Science Herald

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet athttp://dnb.dnb.de

№ 4/2017 – 30
Passed in press in September 2017