Denefil O.V., Bolyukh O.O. Interleukins changes in blood of non-ethanol liver damage rats of different sex. Journal of Education, Health and Sport. 2022;12(8):428-437. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2022.12.08.045
https://apcz.umk.pl/JEHS/article/view/JEHS.2022.12.08.045
https://zenodo.org/record/6984961

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 21, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences).

Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

Received: 10.07.2022. Revised: 07.08.2022. Accepted: 08.08.2022.

INTERLEUKINS CHANGES IN BLOOD OF NON-ETHANOL LIVER DAMAGE RATS OF DIFFERENT SEX

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Abstract

Non-alcoholic liver disease is associated with liver injury, such as steatosis, steatohepatitis, fibrosis and cirrhosis. During their development, inflammation is occure and organs damage develops by help of cytokines influences. The intensity of injury depends on sex.

The aim of the study was to evaluate changes of interleukins in the blood of male and female rats with hepatosis, hepatitis used by overdrinking of glucose.

Material and methods of investigation. The experiments were performed on 60 male and 60 female outbred rats aged from four monthes (start of experiment). Animals were divided into five groups – control 1 (6 monthes old animals), control 2 (8 monthes old animals), glucose 2 monthes (3), glucose 4 monthes (4), and glucose 2+2 monthes (5).

Determined in the blood serum Tumor Necrosis Factor Alpha (TNF-α), Interleukin 1 Beta (IL-1β), Interleukin 4 (IL-4), Interleukin 10 (IL-10).

Results. In 3, 4 and 5 groups we determined increasing of proinflammatory interleukins. When comparing the results of 5 groups with 3 in male rats, lower values of anti-inflammatory interleukins were noted with a reliably insignificant difference in the content of pro-inflammatory interleukins. Interleukins changes were not detected in rats female between groups 5 and 3.

When comparing 3 and 4 groups, an increase in TNF-α and IL-1β, a decrease in IL-4 and IL-10 both in males and in females were determing, which depended on the duration of glucose consumption.

When comparing groups 5 and 4 in males rats, only TNF-α decreased, which can be regarded as a decrease in destructive processes when glucose consumption is stopped. In females, we see a decrease in TNF-α and IL-1β, an increase in IL-4 and IL-10, which may indicate a decrease in the effect of glucose or its long-term effect, since the results of groups 3 and 5 in female rats did not differ.

Conclusion. An increase in the content of pro-inflammatory interleukins and a decrease in anti-inflammatory interleukins was detected after 2 months, 4 months of glucose use, which is maintained for 2 months after the cessation of its use only 2 months and depends on the duration of glucose use. In males, the inflammatory reaction is more than in females.

Key words: cytokines, blood, glucose, rats, sex

Introduction. Non-alcoholic fatty liver disease (NAFLD) is a multifactorial metabolic disorder defined by an abnormal accumulation of lipids in the hepatocytes more than 5% of its weight [1]. It affects 25–30% of the world's population and causes 90% of the morbidity in individuals with diabetes mellitus of type-2 (T_2DM). The increasing prevalence of NAFLD in children and adolescents is even more alarming, with 7% in the general population and

34% among obese children [2]. NAFLD is intimately linked to hepatic steatosis, inflammation, insulin resistance (IR), oxidative stress (OS), and ballooning. HFD significantly induced hepatic steatosis, OS, IR, liver and oxidative enzyme elevation and inflammation in experimental animals [3]. The intake of a high-fat diet (HFD) has long been accepted as a major risk factor for developing NAFLD [4]. HFD and its associated OS promote the synthesis of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) in the liver [5].

TNF-α has a pro-inflammatory, proatherogenic effect, causing endothelial dysfunction. IL-1 provides intercellular interactions that cause an inflammatory response through the expression of acute inflammatory proteins in hepatocytes, activation of neutrophils, action on T-helpers and their stimulation, effects on endothelial, smooth muscle cells, macrophages [6, 7]. Low cytokine content is also important for normal liver regeneration [6].

To reduce the manifestations of NAFLD, the necessary various drugs are administered, which have an anti-inflammatory effect, reduce the signs of insulin resistance, and have antioxidant properties. They include saffron. In expirement rats treated with safranal in ascending order of doses 250 and 500 mg/kg orally for 4-weeks showed a reduction in hepatic lipid's accumulation, liver index, hepatic enzymes, collagen, hepatic oxidonitrative stress markers (like AOPP, MDA and NO_2^-), and raised the levels of catalase (CAT) and superoxide dismutase (SOD) enzymes. Glutathione system components, namely glutathione (GSH), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) levels were also restored in the safranal-treated groups. The reduction in serum TNF-α and IR provided further support to the anti-NAFLD effect of safranal. Moreover, the histopathological images indicated reverse of NAFLD activity score (NAS) through mild fatty degeneration, ballooning and inflammation in hepatocytes of treated groups. [3].

However, not all individuals suffer equally from the development of pathology of the liver during overeting of fats, as it also depends on cabohydates sing and individual reactivity.

The aim of the study was to evaluate changes of interleukins in the blood of male and female rats with hepatosis used by overdrinking of glucose.

Material and methods of investigation. The experiments were performed on 60 male and 60 female outbred rats aged from four months (start of experiment). Animals were divided into five groups – control 1 (6 months old animals), control 2 (8 months old animals), glucose 2 months (3), glucose 4 months (4), and glucose 2+2 months (5).

Control groups were kept on the standard diet of the vivarium during all life with free access to food and water. Group 1 has 6 months and group 2 – 8 months, when they take out from experiment.

Animals of the glucose 2 months group (3 group) were in standard vivarium diet with free access to food, but during 2 months instead of drinking water received a 5 % glucose solution [8].

Animals of the glucose 4 months group (4 group) were in standard vivarium diet with free access to food, but during 4 monthes instead of drinking water received a 5 % glucose solution.

Animals of the glucose 2+2 months group (5 group) were in standard vivarium diet with free access to food, but during 2 months instead of drinking water received a 5 % glucose solution and after that 2 months grinking water.

All animals underwent histological examination of the liver to confirm the simulated pathology.

All experiments were performed in the morning in a specially designated room at a temperature of 18-22 ^{o}C, a relative humidity of 40-60% and an illumination of 250 lux. All animals kept in one room on a standard diet and vivarium regime. The experiments were performed in compliance with the norms of the Council of Europe Convention on the Protection of Vertebrate

Animals Used for Research and Other Scientific Purposes (Strasbourg, 18.03.1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Ministry of Health of Ukraine № 690 of September 23, 2009.

Euthanasia of rats was performed by total bloodletting from the heart after previous thiopental-sodium anesthesia (60 mg/kg, intraperitoneally). For further experimental study in the blood serum were determined the concentration of interleukins [9]. Determination in the blood serum of cytokines levels was performed using by immunopherment method with standard reagent, adapted for white rats “Enzyme-linked Immunosorbent Assay; Kits for Rat: Tumor Necrosis Factor Alpha (TNF-α), Interleukin 1 Beta (IL-1β), Interleukin 4 (IL-4), Interleukin 10 (IL-10)”, Uscn, Life Science Inc., E90133Ra, E90563Ra, CSB–E04635r, CSB–E04595r on the analyzer STAT FAX 303 plus.·

Statistical processing of digital data was performed using the program "STATISTICA" 8.0 ("Statsoft", USA) [10].

Results and discussion. In the control of 6-months-old male rats, compared with female, showed higher by 13.6% ($p<0.01$) values of the pro-inflammatory cytokine TNF-α and higher by 12.5% ($p<0.01$) values of the pro-inflammatory cytokine IL-1β. Anti-inflammatory cytokine have opposite direction: they are higer in females. IL-4 is higher in rats-female, compare to males, by 4.2% ($p<0.05$) and IL-10 is higher by 27.1% ($p<0.001$) (table 1).

Table 1 – The value of interleukins in the serum of rats with the development of non-ethanol damage of liver, M ± m, n = 12

Index	Group	
	Male	Female
Control 1		
TNF-α, pg/ml	4.25±0.14	3.74 ± 0.08**
IL-1β, pg/ml	2.78 ± 0.05	2.47 ± 0.05**
IL-4, pg/ml	7.83 ± 0.08	8.16 ± 0.08**
IL-10, pg/ml	10.14 ± 0.11	12.89 ± 0.12**
Glucose, 2 monthes		
TNF-α, pg/ml	5.87±0.09*	4.42 ± 0.07*,**
IL-1β, pg/ml	3.12 ± 0.06*	2.79 ± 0.04*,**
IL-4, pg/ml	7.48 ± 0.07*	7.76 ± 0.06*,**
IL-10, pg/ml	9.12 ± 0.09*	12.03 ± 0.10*,**
Control 2		
TNF-α, pg/ml	4.42±0.11	3.92 ± 0.08**
IL-1β, pg/ml	2.99 ± 0.06	2.61 ± 0.04**
IL-4, pg/ml	7.58 ± 0.07	7.89 ± 0.07**
IL-10, pg/ml	9.44 ± 0.12	12.43 ± 0.11**
Glucose, 4 monthes		
TNF-α, pg/ml	6.27 ± 0.12*,#	4.79 ± 0.06*,**,#
IL-1β, pg/ml	3.34 ± 0.05*,#	2.96 ± 0.05*,**,#
IL-4, pg/ml	6.97 ± 0.09*,#	7.43 ± 0.08*,**,#
IL-10, pg/ml	8.41 ± 0.12*,#	11.16 ± 0.12*,**,#
Glucose, 2+2 monthes		
TNF-α, pg/ml	5.73 ± 0.14*,##	4.36 ± 0.07*,**,##
IL-1β, pg/ml	3.21 ± 0.04*	2.76 ± 0.03*,**,##
IL-4, pg/ml	7.18 ± 0.06*,#	7.61 ± 0.06*,**
IL-10, pg/ml	8.76 ± 0.11*,#	11.82 ± 0.09*,**,##

Notes: 1. * – indexes are reliable, compared to the age control;
2. ** – indexes are reliable, compared to male rats;
3. # – indexes are reliable, compared to glucose 2 months;
4. ## – indexes are reliable, compared to glucose 4 months.

After drinking glucose instead of water for 2 months, the concentration of pro-inflammatory cytokines increased and anti-inflammatory cytokines decreased in all animals. Level of TNF-α increase in males by 38.1% ($p<0.001$), in females by 18.2% ($p<0.01$). Indexs of IL-1β increase in males by 12.2%

($p<0.01$), in females by 12.9% ($p<0.01$). Concentration of IL-4 decrease in males by 4.7% ($p<0.05$), in females by 5.1% ($p<0.05$). Level of IL-10 decrease in males by 11.2% ($p<0.01$), in females by 7.1% ($p<0.05$). In that group of 6-month-old male rats, compared with female, showed higher by 32.8% ($p<0.001$) values of the pro-inflammatory cytokine TNF-α and higher by 11.8% ($p<0.01$) values of the pro-inflammatory cytokine IL-1β. Anti-inflammatory cytokine have opposite direction: they are higer in females. IL-4 is higher in rats-female, compare to males, by 3.7% ($p<0.05$) and IL-10 is higher by 31.9% ($p<0.001$).

In the control of 8-months-old male and female rats all cytokins increased, but results was not statistic different from 6-months-old male and female rats. In the control of 8-month-old male rats, compared with female, we saw the same directions of result, which was in 6-months-old rats. Rats-male had higher by 12.7% ($p<0.01$) values of the pro-inflammatory cytokine TNF-α and higher by 14.6% ($p<0.01$) values of the pro-inflammatory cytokine IL-1β. Anti-inflammatory cytokine also were higer in females. IL-4 is higher in rats-female, compare to males, by 4.1% ($p<0.05$) and IL-10 is higher by 31.5% ($p<0.001$).

After drinking glucose instead of water for 4 months, the concentration of pro-inflammatory cytokines increased and anti-inflammatory cytokines decreased in all animals. Level of TNF-α increase by 41.8% ($p<0.001$) in males and by 22.2% ($p<0.01$) in females. Indexs of IL-1β increase in males by 11.7% ($p<0.01$), in females by 13.4% ($p<0.01$). Concentration of IL-4 decrease in males by 8.7% ($p<0.05$), in females by 6.2% ($p<0.05$). Level of IL-10 decrease in males by 12.4% ($p<0.01$), in females by 11.5% ($p<0.05$). In that group of 8-months-old male rats, compared with female, showed higher by 30.9% ($p<0.001$) values of the pro-inflammatory cytokine TNF-α and higher by 12.8% ($p<0.01$) values of the pro-inflammatory cytokine IL-1β. Anti-inflammatory cytokine have opposite direction: they are higer in females. IL-4 is higher in rats-female, compare to males, by 6.6% ($p<0.05$) and IL-10 is higher by 32.6% ($p<0.001$).

After drinking glucose instead of water for two monthes and than two monthes drinking water (5 group), the concentration of pro-inflammatory cytokines increased and anti-inflammatory cytokines decreased in all animals. Level of TNF-α increase by 29.6% ($p<0.001$) in males and by 11.2% ($p<0.01$) in females. Indexs of IL-1β increase in males by 7.4% ($p<0.05$), in females by 5.7% ($p<0.05$). Concentration of IL-4 decrease in males by 5.6% ($p<0.05$), in females by 3.7% ($p<0.05$). Level of IL-10 decrease in males by 7.9% ($p<0.05$), in females by 5.2% ($p<0.05$). In 5 group of male rats, compared with female, showed higher by 31.4% ($p<0.001$) values of the pro-inflammatory cytokine TNF-α and higher by 16.3% ($p<0.01$) values of the pro-inflammatory cytokine IL-1β. Anti-inflammatory cytokine increase and was higer in females. IL-4 is higher in rats-female, compare to males, by 6.0% ($p<0.05$) and IL-10 is higher by 34.9% ($p<0.001$).

When comparing the results of 5 groups with 3 in male rats, lower values of anti-inflammatory interleukins were noted with a reliably insignificant difference in the content of pro-inflammatory interleukins. Interleukins changes were not detected in rats female between groups 5 and 3.

When comparing 3 and 4 groups, an increase in TNF-α and IL-1β, a decrease in IL-4 and IL-10 both in males and in females were determing, which depended on the duration of glucose consumption.

When comparing groups 5 and 4 in males rats, only TNF-α decreased, which can be regarded as a decrease in destructive processes when glucose consumption is stopped. In females, we see a decrease in TNF-α and IL-1β, an increase in IL-4 and IL-10, which may indicate a decrease in the effect of glucose or its long-term effect, since the results of groups 3 and 5 in female rats did not differ.

Conclusion. An increase in the content of pro-inflammatory interleukins and a decrease in anti-inflammatory interleukins was detected after 2 months, 4 months of glucose use, which is maintained for 2 months after the cessation of

its use only 2 months and depends on the duration of glucose use. In males, the inflammatory reaction is more than in females.

REFERENCES

1. Samuel V. T., Shulman G. I. Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. Cell Metab. 2018;27(1):22–41.

2. Polyzos S. A., Kountouras J., Mantzoros C. S. Obesity and nonalcoholic fatty liver disease: from pathophysiology to therapeutics. Metabolism. 2019;92:82–97.

3. Reduction of Hepatic Steatosis, Oxidative Stress, Inflammation, Ballooning and Insulin Resistance After Therapy with Safranal in NAFLD Animal Model: A New Approach / U. Sabir, H. M. Irfan, Alamgeer, A. Ullah, Y S. Althobaiti, M. H. Asim // J. Inflamm. Res. 2022;15:1293-1316.

4. Lian C-Y., Zhai Z-Z., Li Z-F., Wang L. High fat diet-triggered non-alcoholic fatty liver disease: a review of proposed mechanisms. Chem Biol Interact. 2020;330:109199.

5. Asrih M., Jornayvaz F. R. Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance. J Endocrinol. 2013;218(3):R25–R36.

6. Molecular mechanisms of neuroimmunoendocrine effects of alcohol / A. N. Ilnitski, N. I. Zhernakoua, L. I. Postnikoua, O. A. Borisov, N. M. Pozdnyakoua // Scientific information. Medicine series. Pharmacy. 2011;4(99),13:5–12. [in Russian].

7. Tumour Necrosis Factor Microsatellite Haplotypes Are Associated with Chronic Pancreatitis / D. O. 'Reilly, S. Dunlop, K. Sargen, A. Demaine, S. Wilkinson, A. N. Kingsnotrh // J. Pancreas. 2006;7(1):14–26.

8. Kostyuk O.A., Denefil O.V., Holovata T.K. Patent № 135342 IPC: G 09 B 23/28; Method for modeling chronic ethanol hepatosis in highly emotional

and low-emotional male rats. Published on June 25, 2019, Bull. 12/2019. [in Ukrainian].

9. Sennikov S. V., Silkov A, N. Methods for determination of cytokines // Cytokines and inflammation. 2005;4(1):22–27. [in Russian].

10. Lapach S.N., Chubenko A.V., Babich P.N. Statistical methods in biomedical research using Excel. Kyiv: Morion, 2000. 320 c. [in Russian].