The effect of rats´ diet to production pro-inflammatory and anti-inflammatory cytokines

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Abstract: The aim of this study was to proof supposed influence of long-term consumption of n-3 PUFA on pro-inflammatory and anti-inflammatory cytokine levels in rats´ plasma. The results will be used to look for consequences to human nutrition. The plasma of experimental rats was examined for levels of IL-10, IL-6, TNF-α, TGF-β and adiponectin. The sources of PUFA n-3 and n-6 were added to standard feed for rats. The experimental animals were divided into four groups. In the standard feed for the first group 6% of safflower oil was added (n-6). The feed ration of the second group contained 6% of fish oil (n-3, eicosapentaenoic acid - EPA). The last group was fed with addition of 6% *Schizochytrium microalgae* oil (n-3, dokosahexaenoic acid – DHA). Control group was fed without addition of oils. Addition of oils didn't change homeostasis of cytokine levels of rats´ plasma.

Key-Words: ELISA, n-3 fatty acids, n-6 fatty acids, IL-10, IL-6, TNF-alfa, TGF-beta 1, adiponectin

Introduction

Atherosclerotic disease cause cardiovascular events and is considered to be a multifactorial condition. Disrupted endothelial homeostasis and infiltration of the intima by activated T cells and monocytes is observed in earlier stages and local production of a variety of inflammatory mediators can modulate and the immunologic reaction within atherosclerotic lesions [7].

Polyunsaturated fatty acids (PUFA) have affected the activity and functional status of blood vessels and process of atherogenesis which caused cardiovascular disease. Eicosanoids (PG2, TA2) are metabolites of PUFA n-6 and they act pro-inflammatory, vasoconstrictor, causing platelet aggregation. On the other side, eicosanoids of PUFA n-3 (PG3, TA3) act anti-inflammatory, vasodilator and anti- platelet aggregation. PUFA n-3 ultimately reduces the risk of cardio- vascular disease, autoimmune diseases and cancer [3]. Cytokines are highly active substances which production is regulated temporally and locally. Its signals lead to change in expression in target cells. Pro-inflammatory cytokines are for example interleukin 6, tumor necrosis factor-alpha, etc. On the contrary anti-inflammatory cytokines are interleukin 10, transforming growth factor-beta, adiponectin, etc. [9]. Interleukin 6 (IL-6) is a cytokine that can facilitate autoimmune phenomena, amplify acute inflammation and promote the evolution into a chronic inflammatory state [1]. IL-6 is produced by smooth muscle cells in the *tunica media* and occurs to the proliferation of these same cells. Persistent inflammation stimulates artery wall remodelling and foam-cell formation, the hallmark of early atherosclerotic lesion. Systemic inflammation may also contribute to atherosclerosis. Indeed serum levels of pro-inflammatory markers positively correlate with the risk of myocardial infarction and cardiovascular death [6] and particularly serum IL-6 levels are directly related to intima-media thickness [5]. Interleukin-10 (IL-10), a cytokine with anti-inflammatory properties, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host [8]. Transforming growth factor-beta 1 (TGF-β1) belongs to transforming growth factor beta family of cytokines which have got a lot of isoforms. TGF-β1 is an anti-inflammatory cytokine. TGF β-1 is a chemoattractant of macrophages and it applies in induction of inflammatory and later in its inhibition. TGF-β 1 is significant for tissue regeneration, induction of IgA and creation of extracellular matrix [9]. Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine and it has an antagonistic activity against TGF-β1. TNF-α is capable of

regulating the activity of cardiac fibroblasts by decreasing collagen synthesis and increasing matrix metalloproteinase activity [4]. Adiponectin is the most abundant adipokine secreted by adipocytes that may couple regulation of insulin sensitivity with energy metabolism. Decreased plasma adiponectin levels are observed in patients with obesity, type 2 diabetes, hypertension, metabolic syndrome and coronary artery disease. Low plasma adiponectin levels are significantly correlated with endothelial dysfunction. These results suggest that low adiponectin levels may be a useful marker for early-stage atherosclerosis [2].

In this study, we have dealt with the impact of income n-3 and n-6 fatty acids on production of pro-inflammatory and anti-inflammatory cytokines in plasma of rats. The aim is tested the hypothesis about effect of long-term consumation of n-3 PUFA on plasma cytokines levels in animal model and apply this knowledge in human nutrition.

Material and Methods

There were added sources of PUFA n-3 and n-6 to standard rat feed ration (Biokron, Czech Republic). Animals were divided into four groups and they were fed with standard diet and diet with 6% addition of safflower oil (n-6), group with 6% addition fish oil (n-3) and group with 6% addition *Schizochytrium microalgae* oil (n-3), which contain docosahexaenoic acid (DHA). The rats were fed for 40 days ad libitum and had ad libitum intake of water. Each group was composed of 10 animals. DHA oil and fish oil are rich in proportion of n-3 fatty acids, safflower oil is rich of n-6 fatty acids.

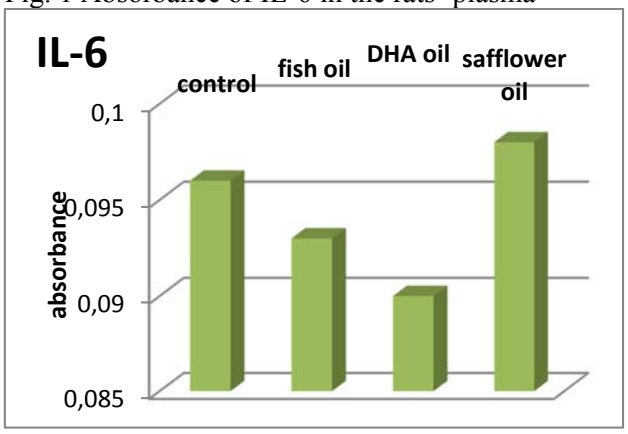
Blood samples were taken from all rats to heparin tubes (DISPOLAB, Czech Republic) at the end of the experiment. From this samples plasma was obtained and analyzed for the concentration of cytokines by ELISA (rat ELISA kits, Invitrogen, USA). Samples were evaluated by Hybrid Reader (Synergy H1, BioTek, USA) at wavelengths 450 nm.

Results and Discussion

Cytokine TGF-β 1 was the only with measurable concentration. Concentrations of other cytokines were so low that they were not detected by ELISA. Absorbance was the only parameter we gained for those cytokines.

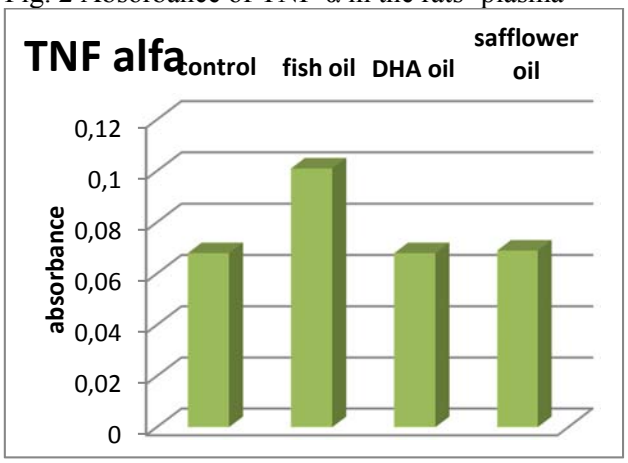
IL-6 is a pro-inflammatory cytokine. According to our hypothesis, its concentration should be highest in the group of rats which was fed a diet containing safflower oil and the lowest concentration in the groups of rats which were fed a diet with fish and DHA oil. The 0.098 average absorbance was determined in a group fed with safflower oil. In the fish oil group average absorbance of 0.093 was determined and the group of DHA oil had a 0.090 average absorbance. In the control group was the average absorbance 0.096. The tendency of absorbance corresponds to our hypothesis but the results are not significant. (Fig.1)

Fig. 1 Absorbance of IL-6 in the rats´ plasma



TNF-α is a pro-inlammatory cytokine. The average absorbance of TNF-α in the control group was 0.068. According to our hypohtesis when the fish and DHA oils have anti-infalmmatory effects, the level of TNF-α in plasma should be lowest in these two groups. It was determined an average absorbance 0.101 in the fish oil group and 0.068 in the DHA group of rats. Absorbance of TNF-α in the fish oil group is higher than in a control group which means that the fish oil induced disruption of homeostasis and increase of pro-inflammatory cytokine level in the rats´ plasma. In the safflower oil group a significant increase of this cytokine was expected. The average absorbance was 0.069 and this is not significant compared to control group. (Fig.2)

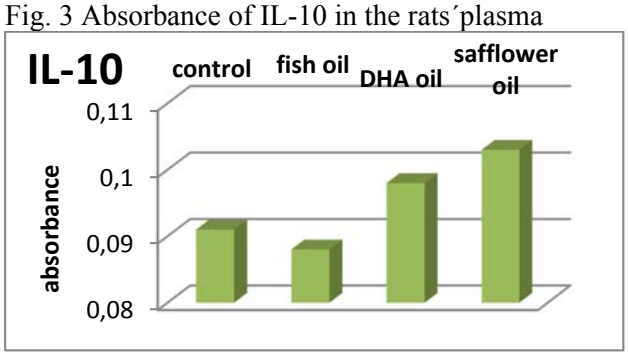
Fig. 2 Absorbance of TNF-α in the rats´ plasma



IL-10 is an anti-inflammatory cytokine. According to our hypothesis the lowest concentration of this

cytokine should be in groups fed with fish and DHA oils addition. In the control group the average absorbance of 0.091 was determined. In the group fed with fish oil the absorbance was 0.088. In the DHA group was determined on average absorbance of 0.098. This value is highert than control. In case of higher concentration IL-10 in the DHA oil group with anti-inflammatory effects means that this type of diet caused changes in homeostasis. There was a suppression of inflammation by IL-10. Average absorbance 0.103 in the group fed with addition of safflower oil corresponds with our hypothesis. Safflower oil with pro-inflammatory effects may induced production of IL-10. Differences of IL-10 levels among fat diets were not significant. (Fig.3)

Fig. 3 Absorbance of IL-10 in the rats´plasma



Average concentration of TGF-β 1 in rats´plasma was 5516.31 pg/mL in the control group. In the group which was fed with addition of fish oil was the average concentration 6380.61 pg/mL. In the group fed with addition of DHA oil average concentraion of the TGF-β 1 was 6453.86 pg/mL. In the group fed with addition of safflower oil the average concentration was 7210.81 pg/mL. These results show that all of these fat diets may caused disruption of homeostasis and production of TGF-β 1 with its pro-inflammatory effects. The results are not statistically significant. (Fig. 4 and Fig.5)

Fig. 4 Absorbance of TGF-β 1 in rats´ plasma

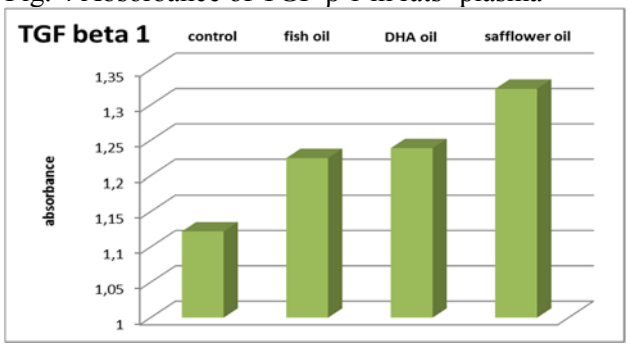
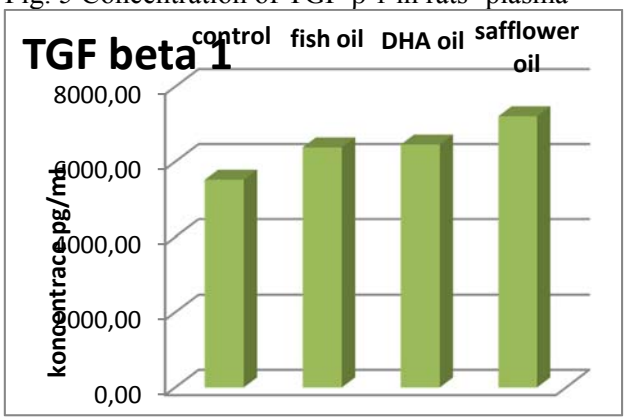
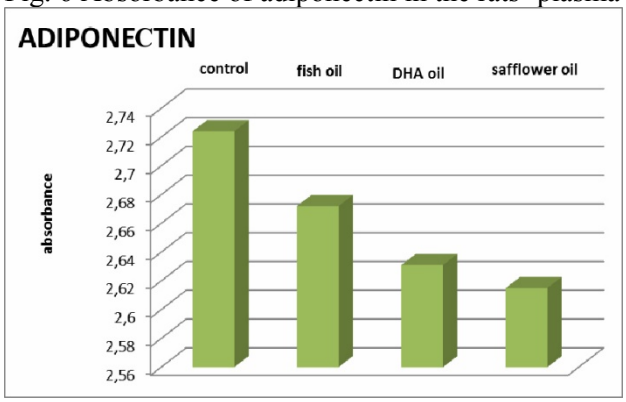


Fig. 5 Concentration of TGF-β 1 in rats´ plasma



The average absorbance of adiponectin in the control group was 2.724. According to our hypothesis the lowest values should be in groups fed with addition of fish and DHA oils. In the group fed with addition of fish oil the average absorbance of adiponectin was 2.672. The group fed with DHA oil had average absorbance 2.631. On the contrary, the highest concentration of adiponectin should be in the group fed with addition of safflower oil to supress the inflammation. This group showed average absorbance 2.651 and it means that fat diet did not disrupt the homeostasis. Results are not significant. (Fig.6)

Fig. 6 Absorbance of adiponectin in the rats´ plasma



Conclusion

No significant results were obtained in this study this supports our hypothesis that DHA and fish oil don´t have any influence of lowering levels of cytokines in rats´ plasma.

Acknowledgments

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