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The Effect of Evening Primrose Oil Supplement into Diet upon the Course of Fever in Rats, Caused by LPS

Wpływ dodatku oleju wiesiołkowego do diety na przebieg gorączki u szczurów wywołanej LPS

In recent years a lot of attention has been paid to therapeutic and dietary properties of evening primrose seeds (*Oenothera biennis* L.), the biological activity of which is connected with the composition of oil extracted from them, which is abundant in unsaturate essential fatty acids (EFA) (6). It is especially relevant concerning linolenic and gamma-linolenic acids belonging to n-6 line, which, owing to existing mechanism of desaturation and elongation, may be transformed into important arachidonic acid being the precursor of the line of physiologically active compounds such as prostaglandins, leucotriens, tromboxane and prostacyclin.

As it is evident from our former studies, supplementing the composition of nutritive dose with evening primrose oil did not result in any visible growth effects in rats, however it modified the activity of some liver enzymes as well as pancreas ones, pointing to the increase in the intensity of intracellular tract of oxidation (1, 4, 5, 8, 10).

Taking into consideration the effects of supplementing diet with isomers of linoleic acid, which results in decrease of lipid peroxidation in mammary gland and inhibition of the development of mammary tumors in rats, we decided to determine the effects of supplementing the diet during 6 weeks by evening primrose oil on the course and the development of fever in rats after endotoxin (LPS) injection.

MATERIAL AND METHODS

The studies were conducted on 24 rats, the Wistar race, of body mass about 235 g. The animals were divided at random into 4 groups of 6 specimens (3 males and 3 females) and were kept in cages, 3 specimens in each, being divided according to sex. The animals were fed for 6 weeks with the mixtures prepared basing on typical fodder laboratory rats LSM (10), varying with 1% evening primrose oil (W group). In the control group (K group), fodder without supplementation of evening primrose oil was applied. During the experiment rats were ensured free access to fodder and water. Fodder and water consumption was checked daily and body mass weekly. 48 hrs before the

termination of the experiment, rats fed with the mixtures supplemented with evening primrose oil (W group) and control rats (K group) were given endotoxin introducing fever. The endotoxin was the one produced in the Cracow Works of Serum and Vaccines, named Pyrogen Standard Kroeger O, Series 10,337, lyophilised lipopolisaccharide (LPS) of *E. coli*. The endotoxine was injected intramuscularly, 0.5 mg/kg body mass, twice every 24 hrs. Body temperature was measured with an electrical thermometer (Ellab Copehagen – Denmark) before and after the application of LPS and at the termination of the experiment.

Next blood for haematological determinations was collected from tail veins of rats and they were etherized and decapitated. During the preparation of animals, livers, stomachs and pancreases were collected. Before preparing, liver was perfused through portal vein with 10 ml of physiological saline and then it was weighted and homogenised in glass Potter homogeniser in 0.06M phosphate buffer of pH = 7.2, whereas for determining superoxide dismutase and alkaline phosphatase also in phosphate buffer, yet of 0.05M and pH = 10.2. Homogenates were centrifuged for 20 min at 13,000 x g. Homogenisation and centrifugation were performed in ice and cooled centrifuges. The activity of isocitrate dehydrogenase (ICDH – E.C.1.1.1.42) (3) and superoxide dismutase (SOD – E.C.1.15.1.1.) (9), asparagine and alanine transaminases (GOT – E.C.2.6.1.1. and GTP – E.C.2.6.1.2.) and also alkaline phosphatase (AP – E.C.3.3.2.1.) (3) as well as protein, were determined in supernatants with the Folin method. In blood, the following parameters were determined: hematocrit value, hemoglobin content, erythrocytes and leukocyte numbers and NBT index with typical tests. The results were subjected to the statistical variance method.

RESULTS AND DISCUSSION

In all the groups animals reached similar body weight after 6 weeks of observation (Tab. 2). No statistically significant differences in the weight of tested inner organs were observed, which indicates lack of visible growth effect of differentiating energetic values of mixtures resulting from supplementing fodder with evening primrose oil.

In livers no distinct and statistically confirmed differences in the activity of

Tab. 1. Composition of mixture

Compound	Content %
Wheat bran	12.5
Corn ground grain	18.5
Wheat ground grain	26.0
Barley ground grain	25.0
Fish flour	4.2
Powder defeated milk	6.2
Fodder yeats	4.2
Fodder chalk	1.3
Fodder phosphate	1.2
Polfamiks LSM	1.0
Protein	15.5
EM (MJ/kg)	11.1

1% evening primrose oil (group W) was added to standard mixture applied to experimental rats.

Tab. 2. Anatomical features of rats ($\bar{x}$ n=6)

Tested feature	Group		
	K	K(LPS)	W(LPS)
Body weight (g)	307	303	287
Body weight increase (g)	65	66	63
Stomach weight (g)	1.7	1.5	1.4
Relative stomach weight (% of body weight)	0.6	0.5	0.5
Liver weight (g)	9.6	9.9	9.7
Pancreas weight (g)	1.0	1.2	1.1

Tab. 3. Enzyme activity (U/mg of protein) ($\bar{x}$ n=6)

Enzyme	Group		
	K	K(LPS)	W(LPS)
Protein mg/g	345	392	316
AspAT (E.C.2.6.1.1.)	274	277	286
AlAT (E.C.2.6.1.2.)	242	246	231
ICDH (E.C.1.1.1.42.)	0.54	0.80	0.75
AP (E.C.3.3.2.1.)	4.10	4.10	4.70
SOD (E.C.1.15.1.1.)	10.73^{A}	7.87^{B}	7.11^{B}

^{A,B} – statistically highly significant differences ($p<0.01$).

Tab. 4. Haematological parameters ($\bar{x}$ n=6)

Feature	Group		
	K	K(LPS)	W(LPS)
Hematocrit (Hct) %	44.6^{a}	40.15^{b}	45.15^{a}
Leukocytes	7783^{a}	6716^{b}	6083^{c}
Neutrophils %	14.5	16.16	12.65
NBT index $\times 10^{-4}$/1 neutrophil	11.78^{a}	12.40^{a}	17.26^{b}
Erythrocytes $\times$ 1000/l	5429	5279	5196
Haemoglobin (Hb – g%)	16.99^{a}	15.40^{b}	14.96^{c}

$^{a, b, c}$ – statistically significant differences ($p<0.05$).

Tab. 5. Body temperature of rats ($\bar{x}$ n=6)

Group	°C		
	measurement I	measurement II	measurement III
K	36.4^{a}	36.6^{A}	36.4
K(LPS)	38.4^{b}	38.5^{B}	37.8^{b}
W(LPS)	37.5^{c}	37.4^{C}	36.5^{c}

$^{a, b, c}$ – statistically significant differences ($p>0.05$).
$^{A, B, C}$ – statistically highly significant differences ($p<0.01$).

asparagine and alanine transaminase as well as alkaline phosphatase were found (Tab. 3). It betokens lack of damage in liver cells as well as their retaining their proper functions. The rats, which were treated with LPS, revealed isocitric dehydrogenase activity increase. The activity of isocitric dehydrogenase of the rats treated with LPS was higher by about 40%. Such difference, although not confirmed statistically, yet suggests certain intensification of catabolic processes in liver. Statistically significant differences in the activity were discovered for superoxide dismutase activity (SOD). Lower SOD activity in animals treated with LPS may betoken the engagement of the processes of liver transformation of lipids to produce active metabolites then used in the cells.

Basic haematological blood values are presented in Table 4. If hematocrit value lower than in the control group, with rats treated with LPS and fed with fodder without fat addition, may be recognised as a typical reaction connected with the heightened intake of water by the animals under the state of fever, yet the quantity of haemoglobin and erythrocytes seems to be also dependent on the nutritive factor, not being directly related to the state of fever. In the rats fed with fodder with the addition of evening primrose oil, almost 22% less leukocytes were found than in the control group. A lower number of this type of blood cells in the first two days of LPS action may manifest more efficient functioning of the immunological system, mainly their penetration outside blood vessels focusing in places of LPS injection (1).

The value of NBT index as compared with the control group was nearly by 43% higher with rats receiving evening primrose oil. On the basis of these results, the conclusion may be arrived at of a higher demand of cells for oxygen and therefore of directing the way of inactivating the antigen against the oxygenous system leading through the so-called oxygen burst, to the creation of bactericidal free hydroxyl radicals, derived mainly from lipid metabolism, consequently leading to the creation of halides such as hypochlorous acid or chloramine (10). Moreover, it should be stressed that with the rats of the control group, LPS application did not cause practically the increase in the NBT index value, which suggests oxygen-free system of suppression of the invasive factor in the situation of lower essential fatty acids supply. The body temperature of these animals was the highest after application of LPS.

The course of body temperatures is presented in Table 5. The strongest reaction against LPS was observed with the rats fed with fodder not supplemented with evening primrose oil. With the rats kept on the diet enriched with evening primrose oil, the course of fever was milder and shorter. After 24 hrs since the application of LPS, body temperature of rats fed with fodder supplemented with evening primrose oil was lower by 1°C than with the ones fed with fodder not enriched with fat. However, the question arises, why the animals fed with fodder supplemented with oil reacted weaker on LPS. The explanation of this phenomenon needs, however, additional studies. Yet, one may suppose

that it results from the intensification of the processes of free radical creation in leukocytes and especially in neutrophils, as the result of oxygenous oxidation of the lipids supplied in fodder. The process seems to be the supplement of the system liquidating the invasive factor, enabling its destruction without the need of complete mobilisation of endogenous pyrogen and therefore only slight changes in raising the temperature of set-point temperature pattern. With the animals fed with fodder not oil-enriched, less effective, anaerobic system of neutralising bacteria is probably enforced, owing to the inadequte radical substrates, and this system causes initiating the whole arachidonic cascade (10), in consequence leading to a more dramatic course of the animals body temperature. This evokes more intensive production and liberation of endogenous pyrogens like interleukin 1 or TNF, consecutively responsible for longer duration and higher change in the hypothalamic set-point temperature.

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STRESZCZENIE

W doświadczeniu przeprowadzonym na szczurach rasy Wistar badano wpływ dodatku 1% oleju z nasion wiesiołka (*Oenothera biennis* L.) na reakcję związaną z domięśniowym podaniem pirogennej endotoksyny LPS z *E. coli*. Statystycznie istotne różnice odnotowano w aktywności wątrobowej dysmutazy ponadtlenkowej (SOD) oraz wskaźników hematologicznych, takich jak: hematokryt, zawartość leukocytów, wskaźnik NBT oraz zawartość hemoglobiny. Przebieg zmian temperatury ciała u badanych szczurów różnił się także w porównywanych grupach żywieniowych. Uzyskane wyniki wskazują na pozytywny wpływ oleju z wiesiołka na reakcje odpornościowe badanych szczurów oraz na niektóre parametry hematologiczne.