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COMPARISON OF RETABOLIL, 4-THIAZOLIDINONE DERIVATIVE (LES-2222) AND TESTOSTERONE ACTION ON THE ACTIVITY OF THE ANTIOXIDANT DEFENCE ENZYMES IN RATS UNDER FOOD DERIVATION

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ABSTRACT: The manuscript devoted to investigation of some patterns of the mode of action of retabolil, compound LES-2222 (new thiopyrano[2,3-d]thiazole derivative (2-(2-Oxo-5a,11b-dihydro-5H,6H-7-oxa-1,4-dithia-3-aza-cyclopenta[c] phenanthren-3-yl)-N-(3-trifluoromethyl-phenyl)-acetamide) – synthesized at the Department of Pharmaceutical, Organic and Bioorganic Chemistry) and testosterone in rats under food deprivation. The changes of the activity of antioxidant enzymes – glutathione peroxidase (GPO) and catalase (CAT) were investigated in the different tissues under food deprivation and compounds treatment. The effects of all tested compounds were similar. The GPO activity decreased under the LES-2222 application in the liver and kidney and was increased in spleen and testes, but in the myocardium and skeletal muscle its level was normalized. Testosterone and retabolil treatment did not change the GPO activity in the liver and spleen. While, the decrease of activity level in kidney and skeletal muscle and normalisation in the myocardium and testes were detected. The changes of CAT activity under the tested compounds action were noticeable, but not statistically significant in the liver and testes. Significantly increased level of CAT activity in the spleen was detected. In the skeletal muscle and myocardium and kidney tissues the enzyme levels were unchanged.

INTRODUCTION: The processes involving molecular oxygen and reactive oxygen species are constantly occurring in the living systems. The latter include the lipid peroxidation (LPO). The formation of free radicals and LPO are the physiological processes under normal conditions and are essential for the realisation of row of functions, such as pinocytosis, phagocytosis, regulation of the membrane permeability, nervous excitement etc.[1-5]

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The intensity of the free radical processes flow depends on the concentration of oxygen in the tissues and the activity of antioxidant enzymes as well as a non-enzymatic antioxidant defence system.

The accumulation of free radicals and reactive oxygen species in the body is a prerequisite and cause of the further oxidative stress, which plays a leading role in the pathology of various origins. Antioxidant defense system (AOS) under the normal conditions prevents the manifestation of harmfull effects of free radicals and peroxides, and regulates the lipid peroxidation reactions in cellular structures. Since AOS system plays a key role in the living systems via regulation of a number of metabolic processes, the evaluation of its state

allows to estimate the quantitative characteristics of these processes progress. Thus, the level of activity of the antioxidant defense system under endogenous and exogenous factors action can be a significant factor in adaptation to environmental changes [6-11].

The aim of the paper was the study of retabolil, compound LES-2222 and testosterone propionate influence on the activity of catalase (CAT) (EC 1.11.1.6) and glutathione peroxidase (GPO) (EC 1.11.1.9) in the different tissues under food deprivation in rats (Compound LES-2222 – thiopyrano[2,3-*d*]thiazole derivative (2-(2-Oxo-5a,11b-dihydro-5*H*,6*H*-7-oxa-1,4-dithia - 3 - aza-cyclopenta[c]phenanthren-3-yl) - N- (3 - trifluoro methyl-phenyl)-acetamide) was synthesized at the Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University). [12-14]

MATERIALS AND METHODS:

All investigative procedures and the animal facilities conformed to the Guide of Care and Use of Laboratory Animals within European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Stratsburg: Counsil of Europe 18.03.1986) and Law of Ukraine "On protection of animals from cruelty" (21.02.2006). For the study 30 male rats (5-6 months) (*Rattus Norvegicus* Var. *Alba*, *Wistar*) were used.

The animals were divided into 5 following groups: control group I – animals received a full ration (n=6); control group II – animals were subjected to the food deprivation (with free access to water) for 9 days (n=6); and three experimental groups: experimental group I – animals were subjected to the food deprivation (with free access to water) and treated with retabolil (nandrolone decanoate, intramuscularly 2 mg/kg, 1 per day for 9 days) (n=6); experimental group II – animals were subjected to the food deprivation (with free access to water) and treated with LES-2222 (intraperitoneally 20 mg/kg, 1 per day for 9 days) (n=6); experimental group III – animals were subjected to the food deprivation (with free access to water) and treated with testosterone propionate (intramuscularly 0.5 mg/kg, 1 per day for 9 days) (n=6).

The enzyme activities were determined according to known methods (catalase activity – Koroluk M. et al.;[15] glutathion peroxidase – Moin V.[16]). Data were analyzed by variation statistics using Student's *t*-test and η (M.O. Plohinsky correlation ratio). All values are expressed as means ± SEM. Differences were considered significant when: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. All used chemicals (except LES-2222) were commercially available.

RESULTS AND DISCUSSION: The GPO and CAT activities changed ambiguous and dependent on the properties of substances and their ability to influence the metabolism of tissues and organs. The GPO activity in the liver of animals of the control group I was 0.10±0,007 μmol/min × mg of protein, in the animals of the control group II – was lower by 30.0% ($p < 0.01$; **Table 1**).

TABLE 1: GPO ACTIVITY IN DIFFERENT ORGANS AND TISSUES

Organs and tissues	GPO activity, μmol/min × mg of protein				
	Control		Experiment		
	Group I	Group II	Retabolil	LES-2222	Testosterone
liver	0.10±0.007	0.07±0,006**	0,07±0,006	0,06±0,003	0,07±0,009
kidney	0.08±0.010	0.12±0.035	0,08±0,013	0,10±0,012	0,11±0,011
spleen	0.24±0.024	0.25±0.029	0,35±0,031#	0,32±0,016	0,33±0,018#
testes	0.13±0.017	0.21±0.010**	0,18±0,015	0,27±0,071	0,21±0,035
myocardium	0.12±0.021	0.17±0.022	0,12±0,013	0,10±0,010#	0,11±0,007#
skeletal muscles	0.65±0.052	1.47±0.258*	1,07±0,142*	1,03±0,206	1,37±0,137***

The differences are significant in comparison with control group * I; and # II.

Probably, there are the depletion of glutathione-phase of the antioxidant defence system, including glutathione level as coenzyme of GPO; decreasing of the reduced form of glutathione and accumulation of the oxidized form and as results the loss of ability to convert the hydrogen peroxide

and organic peroxides under a starvation diet. The GPO activity in the liver tissue under retabolil and testosterone action was the unchanged compared to control group II (food deprivation) and was 0.07 μmol / min × mg of protein. The compound LES-2222 led to decreasing of GPO activity (0.06 mol / μmin × mg of protein) but statistically insignificant. In the renal tissue the GPO activity depended on the conditions of research. Thus, in the kidney of intact animals (control group I) the level was – 0.08±0,010 μmol / min × mg of protein; in the animals with food deprivation (control group II) – 0.12±0,035 μmol / min × mg of protein (higher by 33.4%). Retabolil, compound LES-2222 testosterone led to the decreasing of the enzyme activity level on 33.4, 16.7 and 8.4%, respectively compared to the the GPO level in the animals under the food deprivation.

Herewith, the GPO activity level in the group under retabolil treatment had the same as in intact animals (control group I). However, detected differences in the activity of the enzyme in the kidney of the animals of the experimental and control groups were insignificant. These could indicate the weak influence of the experiment conditions (food deprivation) and tested substances on the GPO activity. The similar differences of GPO activity in heart muscle tissue were detected. The enzyme activity was 0.12 ± 0.021 μmol / min × mg of protein in the intact animals (control group I), while the food deprivation led to increase the activity level by 41.6%.

Application of the tested samples led to decrease of GPO activity: retabolil – by 29.5% compound LES-2222 - by 41.2% ($p < 0.05$) and testosterone - by 35.3% ($p < 0.05$) compared to value in the control group II. In this case, the mentioned values of GPO activity reach the level in the animals kept at the the full diet. Thus, the application of retabolil, compound LES-2222 and testosterone normalized the enzyme activity in the myocardium of rats under the food deprivation.

In the study of the GPO activity in skeletal muscle tissue it was found that the food deprivation led to increase of GPO activity more than 2.2 times ($p < 0.05$) compared to intact animals. Under the starvation diet, the tested substances significantly decreased the activity of the enzyme, but the levels of decreasing were varying: retabolil – 37.3%, LES-2222 – 30.0% and testosterone - only 6.9%, compared to the group (control group II) without compounds action. The activity of GPO in skeletal muscle under tested substances application, compared to intact animals (control group I) was also higher (Table 1). Detected difference between the activity of the enzyme in the intact animals and under retabolil and testosterone action could indicate the increasing of the rate of H_2O_2 formation and probably the intensification of oxidation of the structural components of the muscle cells. Whereas, the compound LES-2222 provided the normalization of the GPO activity, compared to the enzyme level in skeletal muscle of intact animals. Thus, it was shown the downward trend in the level of GPO activity under retabolil and testosterone treatment and food deprivation. the compound LES-2222 normalized the activity of GPO in the skeletal muscle of rats.

In contrast to the mentioned tissues, in the spleen of the animals of control groups the GPO activity was the similar (0.24-0.25 μmol / min × mg of protein), whereas in the animals of experimental groups it was found the increased activity of the enzyme. Maximal increasing of GPO activity was detected in the group with retabolil treatment (at 40.0%, $p>0.05$). Application of testosterone led to less expressed increasing of activity (at 32.0%, $p> 0.05$) and compound LES-2222 provide the similar increasing of activity (at 28.0%, $p> 0.05$) compared to the control group II. Most probably, the tested samples stimulated the activity of enzymatic link of glutathione system. This is due to, likely the higher needs of the blood cells elimination from bloodstream or the intensification of their formation. These processes reflected in the activation of free radical oxidation, including the H_2O_2 formation and thus activation of enzyme.

The effects of tested samples on the activity of GPO in testis tissue were different. Application of retabolil and testosterone under food deprivation did not change the enzyme activity compared to control group II (value within 0.18-0.21 μmol / min × mg of protein). Compound LES -2222 led to increase of GPO activity to 0.27 μmol / min × mg of protein (28.5%). Probably, compound LES-2222

stimulates the enzyme activity in testis tissue, which provides a normal spermatogenesis (genetic material protection) and protection from cytotoxic oxygen products, including H_2O_2.

The different changes of CAT activity in organs and tissues of rats **(Table 2**) were detected under tested compounds actions.

TABLE 2: CAT ACTIVITY IN DIFFERENT ORGANS AND TISSUES

Organs and tissues	CAT activity, µmol/min x mg of protein				
	Control		Experiment		
	Group I	Group II	Retabolil	LES-2222	Testosterone
liver	0.07±0.006	0.08±0.015	0.10±0.012	0.08±0.009	0.12±0.018*
kidney	0.26±0.023	0.28±0.069	0.32±0.053	0.34±0.093	0.28±0.063
spleen	0.63±0.033	0.68±0.035	0.74±0.025*	0.73±0.027*	0.68±0.025
testes	0.27±0.024	0.30±0.029	0.36±0.030*	0.38±0.058	0.32±0.015
myocardium	1.28±0.08	1.67±0.16	1.54±0.21	1.70±0.23	1.86±0.04***
skeletal muscles	0.90±0.096	1.25±0.069*	095±0.060##	0.97±0.104	0.90±0.128#

The differences are significant in comparison with control group * I; and # II.

The activity of CAT was increased under the retabolil and testosterone actions 25 and 50%respectively in the rats' liver compared with animals that are kept on food deprivation (control group II). While under the compoind LES-2222 action, the activity value was similar to the control and lower by 25-50% than animals from other experimental groups. However, the differences were not statistically significant.

The study of enzyme activity in the kidney tissue showed the low CAT activity value in the intact animals (control group I) (0.26 ± 0.023 µmol / min × mg of protein). Almost the same CAT activity levels were in the kidneys of rats under food deprivation (0.28 ± 0.069 µmol / min × mg of protein) and testosterone action (0.28 ± 0,063 µmol / min × mg of protein). Retabolil and LES-2222 led to tendentious increase of the activity of CAT, values in the kidneys of animals of mentioned groups were higher (by 14.2 and 21.4% respectively) compared with the control group II. Probably, the tendency to activation of CAT in the renal tissue under retabolil and LES-2222 treatment caused by the enhanced re-adsorbtion of nutrients in the tubules and, consequently, the increasing of H_2O_2 concentration.

In spleen tissue the similar changes of CAT activity were detected. The enzyme activity was higher under the retabolil and LES-2222 treatment by 8.8 and 7.3% respectively, compared with untreated group. The values of activity levels under tested samples action were higher than in intact animals (retabolil by 17.4% ($p < 0.05$) and LES-2222 – 15.8% ($p < 0.05$)). Thus, retabolil and LES-2222 application led to increasing of CAT activity compared to group under food deprivation. It also may be caused by the accumulation of H_2O_2 in spleen tissue. The enzyme activity was significantly higher in the animals under food deprivation and tested compounds treatment compared with intact animals. This may reflect the activation of the oxidative processes and be caused by the elimination of the blood cells from the bloodstream.

CAT activity in the myocardium of intact animals was 1.28 ± 0.08 µmol / min × mg of protein, while the food deprivation caused the increasing of activity level by 23.4%. Thus, by the food deprivation lead to intensification of H2O2 processes formation in the myocardium tissue and as result to the increasing of activity of the enzyme.

Application of the retabolil and LES-2222 almost does not change the activity of the enzyme, which was within 1.54-1.70 µmol / min × mg of protein.
The application of testosterone provided for the slight increase of the activity of CAT in myocardium (11.3%, $p > 0.05$) compared with the mentioned in rats under the food deprivation. Thus the usage of tested compounds under the food deprivation did not significant change the activity of CAT in myocardium compared to untreated animals. At the same time, the activity level of CAT was higher under retabolil (20.3%), LES-2222 (32.8%) and testosterone (45.3% ($p < 0.001$)) action compared to the intact animals. Increase of the activity of the enzyme could indicate the

prooxidant action of male sex hormone and increase the H2O2 level under food deprivation. On the other, it could indicate the stimulation of antioxidant defense system and enhance of utilization of reactive oxygen species.

Statistically significant ($p < 0.05$) difference between the values of CAT activity in skeletal muscle tissues in the intact animals and animals under the food deprivation was showed. The enzyme activities in the experimental groups were within level of the animals which received a full ration (0.90-0.97 μmol / min × mg of protein), but were lower than the value in the group under starvation diet: under retabolil treatment - by 24.0% ($p < 0.01$), LES-2222 - 22.4% and testosterone - 28.0% ($p < 0.05$). Thus, the use of tested substances under the food deprivation inhibited the accumulation of H_2O_2 in rat skeletal muscle tissue, which, respectively, was reflected in the CAT activity inhibition.

In the testes of the animals of the control groups it was found the lowest level of CAT activity (0.27 ± 0.024 – 0.30 ± 0.029 μmol / min × mg of protein) compared with other organs. The application of retabolil, LES-2222 and testosterone led to the increase of CAT activity by 20.0%, 26.6% and 6.6% respectively compared with untreated animals (control group II). However, the differences were statistically insignificant ($p > 0.05$).

Another results are followed after the analysis of enzyme activity in testes of animals under compounds action and starvation when compare with intact animals. The value of CAT activity was significantly higher by 33.3% ($p < 0.05$) under retabolil action. Probably, only retabolil via stimulation of CAT activity with lowering of GPO activity provides normal spermatogenesis and H_2O_2 utilisation.

CONCLUSIONS: The 9-day food deprivation in rats led to the changes of the activity of the enzymes of the antioxidant defence system. The increase of the activity of glutathione peroxidase in the different tissues was shown. While, decrease of GPO activity was observed only in the liver tissue the. Changes of catalase activity were less pronounced – a slight increase of activity level was discovered in all studied tissues. The patterns of the mode of action of all tested compounds (retabolil, testosterone and thipyrano[2,3-*d*]thiazole – LES-2222) were similar. Under the food deprivation all tested compounds led to decrease of enlarged levels of GPO activity in tested tissues, exept spleen tissue. In spleen tissues the increasing of enzyme activity was observed. The CAT activity was more expressed under compounds action. Only skeletal muscle tissue was caracterized by decreased level of CAT activity (comparable with level of activity in intact animals) under the food deprivation and compounds treatment.

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