ORIGINAL ARTICLE

Characteristics of 2-(tetrazol[1,5-c] quinazolin-5-ylthio) acetate effect on the level of adenine nucleotides in intense physical activity

Olena Toziuk[1], Nataliya Voloshchuk[2], Tamara Germanyuk[1], Olena Kryvoviaz[1], Serhii Kryvoviaz[3], Tanya Ivko[1], Andrii Saienko[2]

[1]Department of Pharmacy, National Pirogov Memorial Medical University, Vinnytsya, Ukraine, [2]Department of Pharmacology, National Pirogov Memorial Medical University, Vinnytsya, Ukraine, [3]Department of Eye diseases, National Pirogov Memorial Medical University, Vinnytsya, Ukraine

Abstract

Introduction: We investigated changes in the energy state of the skeletal muscle cells in rats under physical load (15 days). **Materials and Methods:** The experiments were performed on nonlinear white rats. On the 15th day of the experiment, the rats were loaded with running at a speed of 42 m/min and slope angle 10°. The test substance sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28) at a dose of 1.7 mg/kg and reference compound 2-ethylthiobenzimidazole hydrobromide (32.0 mg/kg) were administered to animals intraperitoneally 60 min before treadmill training. Concentration of adenine nucleotides (ATP, ADP, and AMP) was determined in skeletal muscles by thin layer chromatography test. **Results and Discussion:** It was first found that a course of intraperitoneal injection of sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28) contributed to 88.5% ($P < 0.05$) growth of physical endurance. We proved, that unlike the trained control, administration of compound KB-28 prevented imbalance in the system of adenine nucleotides. It was indicated by a significantly important increase of the level of ATP by 54.8% while parallel reducing concentration of ADP and AMP by 16.8 and 43.4%, respectively. **Conclusions:** Restoration of balance in the adenine nucleotides system may be one of the components of sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28) actoprotective effect.

Key words: 2-ethylthiobenzimidazole hydrobromide, actoprotectors, physical endurance, adenine nucleotides, sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio) acetate

INTRODUCTION

Physical performance is not only a criterion for assessing human health but also a key to adaptation to changing environmental conditions. Performing any kind of physical work inevitably leads to fatigue-a set of temporary changes in the physical and mental state of an individual, developing as a result of activities and leading to reduction of quantitative and qualitative indicators. This process is protective in nature, since it prevents a body from full exhaustion through economization of all processes and preservation of physiological reserves.[1-4]

The rate of fatigue is significantly influenced by metabolic changes that occur in physically loaded body. We know that to maintain contractile function of muscles, they should be continuously provided with energy in the form of ATP.[5-7] In this case, synthesis of the latter should occur at a rate corresponding to the intensity of the work performed. According to the reference data, a decrease in energy production occupies one of the leading places among factors that limit physical performance.[1,5]

Address for correspondence:
O. Toziuk, Department of Pharmacy, National Pirogov Memorial Medical University, Vinnytsya, Pirogov Street 56, 21018, Ukraine. Phone: +38-0974487419.
E-mail: olena.tozyuk@gmail.com

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Continuous strenuous physical load without proper rest, which contributes to stabilization of homeostasis and recovery of energy supply, leads to functioning of the body at the edge of physical capacity, which in turn may trigger the emergence of various diseases. Therefore, to preserve human health, substances able of maintaining a high level of work efficiency, preventing fatigue and speeding up the recovery process are proactively searched today among the various classes of chemical compounds.[2,8] The compounds with such properties are known under the title "actoprotectors"–substances of anti-exhaustion type of action intended for maintaining high motor activity in extreme conditions and increasing work efficiency.[9] Promising in this respect is considered quinazoline derivatives with antihypoxic, neuro- and cardioprotective effects, which also play an important role in the high effectiveness of physical work.[1,3-5]

Sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio) acetate (compound KB-28) appeared to be a leader in the previous studies of actoprotective activity of new derivatives of 5-R-thio-tetrazol[1,5-c]quinazoline in normal and complicated experimental conditions.[8,10] The above-mentioned substances were synthesized at the Department of Organic Chemistry of Zaporizhia State Medical University under control of prof. S. I. Kovalenko.[11] The ability of compound KB-28 to enhance physical endurance determines the prospects of deeper research of the substance feasibility as a new actoprotective agent. The literature sources suggest that the capacity of standard actoprotector 2-ethylthio benzimidazole hydrobromide (2-ETBH) to improve energy supply to the human body relates to its positive influence on energy supply indicators of the loaded human body, including the ability to sustain the process of ATP synthesis.[9,12] Taking into account these data, we have come to the need of studying possible mechanisms of actoprotective action of compound KB-28.

Objective of the Study

The objective of the study was to characterize the influence of sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28) at the level of adenine nucleotides in skeletal muscle of rats on the background of the regular physical load as a possible mechanism of its actoprotective action.

MATERIALS AND METHODS

The experiments were performed on 56 white rats of Wistar strain, obtained from the vivarium of the National Pirogov Memorial Medical University, Vinnytsya. The duration of quarantine was 2 weeks. The animals were kept on a standard diet with free access to water and feed with a natural day and night regimen. The animals were fed a semi-synthetic starch-casein diet with a balanced content of all macro- and micronutrients. Individually marked animals, selected after quarantine, were divided into groups of 14 animals each with homogenous body weight (± 15%). All interventions were carried out in compliance with the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), as evidenced by the opinion of the bioethics commission of National Pirogov Memorial Medical University, Vinnytsya (Minutes No. 7 dated 24 April 2014). As a reference substance, we used actoprotector 2-ETBH.

Rats were divided into 4 groups of 14 animals each: (1) intact rats; (2) rats subjected to training load without correction (trained control); (3 and 4) rats, dosed 1.7 mg/kg of compound KB-28 and 32.0 mg/kg of reference substance 2-ETBH, respectively, on the background of regular physical load. The substances, dissolved in isotonic sodium chloride solution, were administered to animals intraperitoneally 60 min before treadmill training. These substances were used in mean effective doses (ED_{50}). ED_{50} were calculated graphically by Litchfield-Wilcoxon method based on the results of the swimming test in the normothermia conditions.[8] The control group of rats received intraperitoneally the equivolume 0.9% sodium chloride solution.

Rats from 2 to 4 groups were loaded daily 5 min for 14 days in a treadmill at a speed of tape 28 ± 1.0 m/min and track angle 10°. Loading animals in a treadmill enable to adjust the intensity of work, and this regime is considered most appropriate for small laboratory animals.[13] In addition, such loads correspond to the high level of intensity of physical work, and it is performed by activating the aerobic-anaerobic energy path, which in this case was the most appropriate for assessment of metabolic changes in the test animals.[1,7,14]

On the 15th day of the experiment, the rats from 2 to 4 groups were loaded with running at a speed of 42 m/min and slope angle 10°. In the first phase of the experiment (half rats from 2 to 4 groups, 7 subjects from each group), we investigated the physical capacity against duration of the race until complete exhaustion, confirmed by no reaction to stimulation with electrical charges (40 V) at the starting line of a treadmill.[13] The remaining 7 animals from groups 2 to 4 (2nd phase of the experiment) were loaded with treadmill running under the same conditions for 10 min 3–5 min after exercise all the rats from the 2nd phase of experiment and the intact rats (Group 1) were taken samples of biological material for the study of energy metabolism indicators.

The biological material (fragment of femoral muscle) was sampled after the decapitation of animals under thiopental anesthesia. Concentration of adenine nucleotides (ATP, ADP, and AMP) was determined in skeletal muscles by thin layer chromatography test.[15] The results were calculated using the standard curve and presented in mmol/g tissue. The energy charge was calculated using D. E. Atkinson formula.[16]

The energy charge = 2 ATP+ADP/2(ATP+ADP+AMP).

Table 1: Effect of KB-28 and 2-ETBH on level of adenine nucleotides in skeletal muscles of rats on the background of daily treadmill training, (M±m, *n*=7)

Indicators	Intact rats	After physical load		
		Trained control	KB-28	2-ETBH
ATP (mmol/g tissue)	3.72±0.29	2.30±0.19*	3.56±0.24#	3.24±0.18#
ADP (mmol/g tissue)	0.83±0.06	1.07±0.08*	0.89±0.05#	0.92±0.06#
AMP (mmol/g tissue)	0.176±0.02	0.338±0.02*	0.188±0.01#	0.194±0.02#
Energy charge	0.875±0.006	0.765±0.009*	0.863±0.004#	0.849±0.007*#

*P<0.05 relative to intact group; #P<0.05 relative to control; *n*: Number of animals in a group from the 2nd phase of experiment. 2-ETBH: 2-ethylthiobenzimidazole hydrobromide

RESULTS AND DISCUSSION

We processed the digital data obtained from the study by variation statistics technique using IBM SPSS Statistic 22 software, calculated mean value M, the arithmetic mean error m, t-Student's criterion for normal distribution, White nonparametric criterion–if the latter was not available, and Wilcoxon (paired samples) criterion–to determine changes in the trend within the group. The changes of parameters were considered significant at $P < 0.05$.

Discussion

In the first phase of the experiment, on the 15th day of the study, we evaluated the physical endurance of rats after daily training. See the results in Figure 1.

It was established, that the length of the run of rats from the trained control group averaged 20.8 min. The course of compound KB-28 and 2-ETBH treatment amid daily training contributed to the significant growth of physical endurance compared to the control group on the 15th day of the experiment. For example, when administered compound KB-28, the length of treadmill run increased against the control animals by 88.5% ($P < 0.05$). Physical performance of rats, injected 2-ETBH in the given conditions of the experiment, increased by 72.1% ($P < 0.05$).

These data suggest that both compound KB-28 and the reference substance reveal actoprotective effect. Compound KB-28 at a dose of 1.7 mg/kg was found not inferior to 2-ETBH at a dose of 32.0 mg/kg in terms of effect manifestation and was 20 times superior to the latter in terms of activity since caused a similar effect with 20 times lower dose.[8]

See the results of the second phase of an experiment in Table 1.

We found that daily running stress in rats caused significant changes in the composition of adenine nucleotides in skeletal muscles compared to intact animals on the 15th day of the study. The rats from the control group (no-correction run) presented significant reduction of ATP content by an average

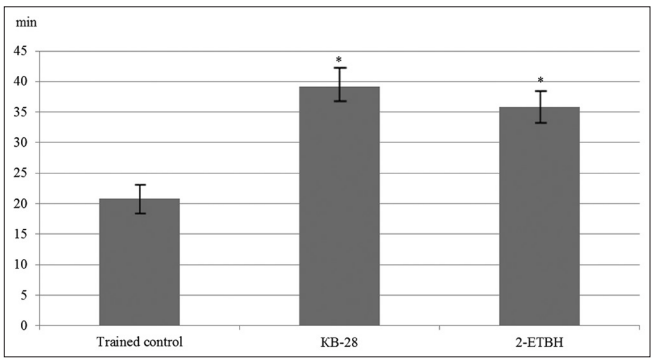

Figure 1: Effect of compound KB-28 and 2-ethylthiobenzimidazole hydrobromide of length of a rat run in treadmill on day 15 of exercise. *$P < 0.05$ relative to trained control

of 38.0%, accompanied with simultaneous growth of ADP and AMP concentration compared to the intact group by 29.0 and 92.0% ($P < 0.05$), respectively. The value of this indicator decreased to 0.765 in the control group rats [Table 1].

Prophylactic administration of compound KB-28 (1.7 mg/kg) to rats before dosed physical activity, unlike animals from the control group, contributed to weakening negative changes in energy supply. This fact manifested in a normalized pool of adenine nucleotides in skeletal muscles of animals. For example, the content of ATP in muscles significantly increased compared to control animals by 54.8% with a certain decrease of ADP and AMP levels. Recovery of adenine nucleotide content in skeletal muscles of rats influenced by study substance was accompanied by the growth of muscle energy charge, which value approached to the level of intact animals [Table 1].

The course of intraperitoneal 2-ETBH treatment at a dose of 32 mg/kg also caused positive changes in comparison with the animals from the control group; the concentration of ATP increased by 40.9% and the energy charge–up to 0.849, but this index was still remaining significantly lower than in the intact group. Compound KB-28 was compared with 2-ETBH against the ability to normalize the pool of adenine nucleotides and was found superior in terms of energy charge, which numeric value restored to the level of intact rats under the influence of the substance. It also should be noted, that

KB-28 is much more active since its ED_{50} is 20 times less than ED_{50} of 2-ETBH.

According to the literature,[1,5,6] such changes in macroergs of the control animals may be attributed to separation of oxidation and phosphorylation processes in the respiratory chain while performing an intense physical activity, which causes cell respiration blocking and rapid development of energy deficit. Under such conditions, dephosphorization of ADP to ATP appears impossible, and further increase of ADP and AMP muscle content usually contributes to fatigue development.[6,7] The energy imbalance in animals on a background of intense exercise has been also experimentally confirmed by changing cell energy charge.[16] It is known that this indicator characterizes the metabolism of a cell and normally ranges within 0.85–0.90. Decrease this indicator is below the specified level regarded as critical for the life of the cell.[1,5]

Therefore, the results of the study revealed the ability of compound KB-28 to improve physical performance of rats under training load, similar to reference actoprotector 2-ETBH. The above-mentioned effect of the test compound was driven by the ability to maintain the proper level of energy supply in the body. Daily intraperitoneal administration of sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28) during 15 days contributed to the adaptive restoration of metabolic processes in favor of increased synthesis of ATP macroerg. The obtained data may indicate the ability of the study substance to economize energy processes and fully use macroergic substrates under exhausting physical activities in a way similar to the reference drug. The literature sources suggest,[9,12] that the ability of 2-ETBH to improve energizing of cells is preconditioned by its activating effect on gluconeogenesis and increased glucose uptake by cells. The results obtained correlates with the results of the study of antioxidant parameters in the body of animals with physical activity.[17] It has been found that the substance helps increase the activity of antioxidant system (reduced glutathione) and provides timely disposal of secondary oxidation products (malonic dialdehyde). It may be assumed, that revealed changes in the system of energy supply is one of the components of the actoprotective effect of sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28).

CONCLUSIONS

Compound KB-28 improves physical performance of rats under training load during 15 days. Daily intraperitoneal administration of sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28) contributed to the adaptive restoration of metabolic processes in favor of increased synthesis of ATP macroerg.

It may be assumed, that revealed changes in the system of energy supply is one of the components of the actoprotective effect of sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate (compound KB-28).

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