12. Hougeir, F. G., Kircik, L. (2012). A review of delivery systems in cosmetics. Dermatologic Therapy, 25 (3), 234–237. doi: 10.1111/j.1529-8019.2012.01501.x

13. Vigan, M., Castelain, F. (2014). Cosmetovigilance: definition, regulation and use "in practice". European Journal of Dermatology, 24 (6), 643–649.

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INVESTIGATION OF EFFECT OF SODIUM 2-(TETRAZOL[1,5-c]QUINAZOLIN-5-ILTIO)ACETATE (COMPOUND KB-28) ON THE METABOLIC PROCESSES IN ORGANISM DURING INTENSIVE EXERCISE

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Виконання інтенсивних фізичних навантажень супроводжується рядом метаболічних змін у організмі. Одними з них є активація системи вільнорадикального окиснення та зниження антиоксидантного захисту, тобто виникнення оксидативного стресу. Задля підтримки високого рівня працездатності та утилізації побічних продуктів обміну доцільно використовувати фармакологічні засоби з групи актопротекторів.

***Метою** даної роботи було дослідити вплив натрію 2-(тетразоло[1,5-с]хіназолін-5-ілтіо)ацетату (сполуки КВ-28) на прооксидантно-антиоксидантний баланс в організмі щурів за умов інтенсивних фізичних навантажень.*

***Матеріали та методи.** Для вивчення метаболічних процесів у печінці щурів за умов інтенсивних фізичних навантажень було обрано модель бігу в тредбані. Протягом 14 діб щурів тренували у тредбані при швидкості руху стрічки 28±1,0 м/хв та куті нахилу 10°. На 15-ту добу експерименту тварин навантажували бігом (швидкість руху доріжки 42 м/хв, кут нахилу 10°) протягом 10 хв і забирали біологічний матеріал для біохімічного дослідження. Вплив речовин на активність прооксидантно-антиоксидантної системи визначали за вмістом у гомогенаті печінки відновленого глутатіону та продуктів, що реагують з тіобарбітуровою кислотою.*

***Результати.** Щоденне введення в організм щурів сполуки КВ-28 в умовах інтенсивних фізичних навантажень сприяло зниженню у гомогенаті печінки вмісту продуктів, що реагують з тіобарбітуровою кислотою, на 23,0 % та підвищенню рівня відновленого глутатіону на 60,0 % відносно контролю. Це вказує на спроможність сполуки КВ-28 нормалізувати прооксидантно-антиоксидантний баланс, порушення якого є одним із факторів, що лімітують фізичну працездатність.*

***Висновки.** Стабілізація прооксидантно-антиоксидантного балансу на тлі дії сполуки КВ-28 може бути одним із механізмів реалізації її актопротекторної та стреспротективної дії*

***Ключові слова:** актопротектори, інтенсивні фізичні навантаження, натрію 2-(тетразоло[1,5-с]хіназолін-5-ілтіо)ацетат, 2-етилтіобензімідазолу гідробромід, прооксидантно-антиоксидантний баланс*

Implementation of intense exercise is accompanied by a number of metabolic changes in organism. One of them is the activation of free radical oxidation and reduction of antioxidant protection that is oxidative stress occurrence. To maintain high level of efficiency and utilization of by-products of metabolism, the use of actoprotectors is appropriate.

*The **aim** of the given research was to investigate the effect of Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate (compound KB-28) in the prooxidant-antioxidant processes in rats under conditions of intense exercise.*

Materials and methods. *To study metabolic processes in the liver of rats under conditions of intense exercise, the model of running in a treadmill was chosen. During 14 days, the rats were trained in the treadmill at tape speed 28±1,0 m/min and angle 10°. On the 15th day of the experiment the animals were loaded by running (tape speed 42 m/min, the angle 10°) for 10 minutes and the biological material for biochemical research was taken. The influence of substances on the activity of prooxidant-antioxidant system was determined by the content in homogenate of liver glutathione and products that react with thiobarbituric acid.*

Results. *Daily injection of compound KB-28 in rats organism under conditions of intense exercise helped to reduce by 23,0 % in liver homogenate the content of products that react with thiobarbituric acid, and increase the level of glutathione by 60,0 %, compared to control. This indicates the ability of the compound KB-28 to normalize prooxidant-antioxidant processes, which is one of the factors limiting physical capacity.*

Conclusion. *Stabilization of the prooxidant-antioxidant processes under the compound KB-28 action may be one of the mechanisms for implementation of its actoprotective and stress-protective action*

Keywords: *actoprotector, intense exercise, Sodium 2-(tetrazol[1,5-c]quinazolin-5-ylthio)acetate, 2-Ethylthiobenzimidazol hydrobromide, prooxidant-antioxidant processes*

1. Introduction

Stress is a nonspecific reaction of human organism to the influence of any external factors [1]. Physical exercises are one of the strongest adaptive factors that inherently accompany human's life. In response to stressors, mobilization of structural, energy and information resources occurs in human organism, which eventually leads to structural trace formation of and increases organism's resistance.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Implementation of intense exercise under normal and complicated conditions leads to free radical oxidation activation and reduction of antioxidant protection in human organism, which is oxidative stress occurrence [2–6]. In turn, this is accompanied by physical ability decrease or total disability, as well as it may cause the adaptive mechanisms disruption. According to the literature data [1, 7], it was found, that the process of long term adaptation is one of the main ways to optimize working capacity both under normal and complicated conditions. Despite more than half-century history of stress influences and adaptation research, this question remains problematic, due to it concerns the whole planet population regardless of racial and territorial belonging.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

Sufficient amount of energy, oxygen provision and rapid by-products of metabolism utilization are necessary to maintain high level of working capacity [7]. Pharmacological products are advisable to use for this purpose. Actoprotectors are special remedies, able to maintain high physical activity under extreme conditions and increase working capacity, and promising for physical efficiency correction [8, 9]. The group of these remedies is rather small and is represented practically by one 2-Ethylthiobenzimidazol hydrobromide (2-ETBI), which also is not registered in Ukraine. The actoprotective effect of 2-ETBI is realized mainly by the ability to normalize violated metabolic processes during physical work [9]. That is why nowadays the active search of new high-effective substances for the national actoprotector creation is carried out.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

Our attention was drawn to the new 5-R-thiotetrazole[1,5-c] quinazoline derivatives, synthesized in Organic and bioorganic chemistry department of Zaporozhye state medical university under the guidance of prof. S.I.Kovalenko. In screening studies of the mentioned substances, the leader compound Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate (compound KB-28), which clearly increased the physical endurance in rats under normal and complicated conditions of the experiment, was found [10]. Besides, the presence of stress-protective action of KB-28 was determined, that can be considered as one of the links in the adaptation process [11].

The important goal of any experiment is the establishment of mechanisms of action of the studied substances. That provides an opportunity to evaluate better their pharmacological activity and to predict new potential effects. When studying possible mechanisms of Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate (KB-28) actoprotective action, it became necessary to determine its influence on metabolism in organism during intensive physical exercises, including lipid peroxidation and antioxidant protection processes.

5. Formulation of goals (tasks) of Article

The aim of the given research was to investigate the effect of Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate (compound KB-28) in the prooxidant-antioxidant processes in rats under conditions of intense exercise.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

To study metabolic processes in the liver of rats under conditions of intense exercise, the model of running in a treadmill was chosen. The study was carried out in nonlinear rats weighing 180–210 g in accordance to the "General ethical principles of experiments in animals" (Ukraine, 2001), harmonized with the "European convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986). Laboratory animals were kept under standard diet of water and meal and with free access to food and water and under natural changes of day and night.

The rats were randomly divided into 4 groups:

1 – intact rats;

2 – rats after exercises without correction (control group);

3 – rats treated with compound KB-28 (1,7 mg/kg) on the background of exercises;

4 – rats treated with 2-ETBI (32,0 mg/kg) on the background of exercises.

KB-28 and 2-ETBI compounds were used in the doses corresponding to their ED50 according the swimming test. Both the studied substance and the reference compound were dissolved in isotonic Sodium chloride solution and administered intraperitoneally (i/p) every day 60 minutes before training in a treadmill. The control group of rats had an equal amount of pure solvent i/p.

The rats of the 2–4 groups were trained daily in a treadmill during 5 minutes within 14 days at tape speed 28±1,0 m/min and angle 10° [12]. On the 15th day of the experiment the animals were loaded by running at tape speed 42 m/min, the angle 10° for 10 minutes; in 3–5 minutes after the loading they were taken out of the experiment under thiopental anesthesia, and the biological material for biochemical research was taken. The influence of the compounds on the prooxidant-antioxidant system activity was determined by the content of products, reacting with thiobarbituric acid (TBA-AP) [13], and reduced glutathione (RG) in liver homogenate.

The data processing of the given research was carried out by variation statistics method using IBM SPSS Statistic 22 software; arithmetic mean value M, the arithmetic mean error m, t- Student test in the normal distribution, nonparametric White criterion W – in case of its absence, Wilcoxon signed-rank test Ť – were calculated to establish changes in the dynamics inside the group. The indexes changes at $p<0,05$ were considered as reliable [14, 15].

Results of the study of influence of Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate (compound KB-28) and 2-ETBI under intense exercise on the prooxidant-antioxidant balance are given in Table 1.

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Table 1

The influence of KB-28 and 2-ETBI on the prooxidant-antioxidant balance under intense exercise (M±m, n=7)

Indexes	Intact group	Control group	Loading+KB-28	Loading+2-ETBI
TBA-AP (mmol/g tissue)	84,4±5,6	128,3±7,2*	98,8±5,3*#	107,6±8,8*
RG (mmol/g tissue)	7,42±0,36	4,26±0,37*	6,82±0,51#	6,31±0,38*#

*Notes: probable value concerning: * – intact group, $p<0,05$; # – control group, $p<0,05$; n – number of animals in group*

It was found, that sub maximal intensity work without correction (the control group) causes significant prooxidant-antioxidant balance changes. A sign of this was a significant TBA-AP increase in liver by 52 % with simultaneous increase the level of RG by 42,6 %, compared to the intact animals (see the Table). The mentioned TBA-AP and RG changes are consistent with the literature data about oxidative stress occurrence after intense exercise [2–6].

After the influence of compound KB-28, under the mentioned experimental conditions, reduce by 23,0 % in liver homogenate the content of TBA-AP, and increase the level of reduced glutathione by 60,0 %, compared to control was observed. At daily administration of 2-ETBI in rats organism under intense exercise conditions, a certain decrease of TBA-AP level by 16,1 % compared to control was observed. Nevertheless, the given index did not reach the probable values ($p>0,05$). At the same, a significant increase of RG content by 48,1 % compared to control in liver homogenate was observed. It should be noted that the action of compound KB-28, unlike 2-ETBI, was more effective, because decrease of TBA-AP level after its administration was statistically probable compared to control, and the content of RG was on the level of intact animals.

The specified dynamics of TBA-AP and RG levels under KB-28 and reference compound influence points to their ability to normalize prooxidant-antioxidant balance, which violation is one of limiting work capacity factors. The ability of KB-28, as well as 2-ETBI, to provide antioxidant protection and inhibit lipid peroxidation processes in organism under intensive exercises can become one of adaptive mechanisms improving endurance.

It should be also mentioned, that according to the given research results, Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate in the dose of 1,7 mg/kg was more effective than 2-ETBI (32,0 mg/kg), because the reduce of TBA-AP as the final product of lipid peroxidation after its administration was statistically probable compared to control, and RG content reached the intact group level. The mentioned dynamics of TBA-AP and RG concentrations on the background of KB-28 effect can become one of mechanisms of its actoprotective and stress protective action, which was found before.

7. Findings from the research and prospects of further development of this area

1. According to the carried out research it was found that regular intense exercises in rats in a treadmill during 15 days lead to prooxidant-antioxidant processes imbalance.

2. Durable administration of Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate (compound KB-28) and 2-ETBI under the mentioned experimental conditions promotes metabolism optimization in rats organism through antioxidant system activity increasing, which provides forehanded neutralization of oxidation by-products.

3. Sodium 2-(tetrazol[1,5-c]quinazolin-5-iltio)acetate in the dose of 1,7 mg/kg, unlike 2-ETBI (32,0 mg/kg), was more effective, because the reduce of TBA-AP level after its administration was statistically probable compared to control, and RG content reached the intact group level.

4. Normalization of prooxidant-antioxidant balance after compound KB-28 administration can become one of mechanisms of its actoprotective and stress protective action.

References

1. Sel'e, G. Stress bez distressa [Stress without distress] [Text] / G. Sel'e; E. M. Kreps (Ed.). – Moscow: Progress, 1982. – 124 p.

2. Nikolaidis, M. G. Redox biology of exercise: an integrative and comparative consideration of some overlooked issues [Text] / M. G. Nikolaidis, A. Kyparos, C. Spanou, V. Paschalis, A. A. Theodorou, I. S. Vrabas // The Journal of Experimental Biology. – 2012. – Vol. 215, Issue 10. – P. 1615–1625. doi: 10.1242/jeb.067470

3. Morales-Alamo, D. Free radicals and sprint exercise in humans [Text] / D. Morales-Alamo, J. A. L. Calbet // Free Radical Research. – 2014. – Vol. 48, Issue 1. – P. 30–42. doi: 10.3109/10715762.2013.825043

4. Stankovic, M. Oxidative stress and physical activiti [Text] / M. Stankovic, D. Radovanovic // Sportlogia. – 2012. – Vol. 8, Issue 1. – P. 1–11. doi: 10.5550/sgia.120801.en.001s

5. Ji L. L. Antioxidant and anti-inflammatory effects of exercise: role of redox signaling [Text] / L. L. Ji, Y. Zhang // Free Radical Research. – 2013. – Vol. 48, Issue 1. – P. 3–11. doi: 10.3109/10715762.2013.844341

6. Pingitore, A. Exercise and oxidative stress: Potential effects of antioxidant dietary strategies in sports [Text] / A. Pingitore, G. P. P. Lima, F. Mastorci, A. Quinones, G. Iervasi, C. Vassalle // Nutrition. – 2015. – Vol. 31, Issue 7-8. – P. 916–922. doi: 10.1016/j.nut.2015.02.005

7. Farmakologija sporta [Sports Pharmacology] [Text] / S. A. Olejnik, L. M. Guninoj, R. D. Sejfulla (Eds.). – Kyiv: Olimpijskaja literatura, 2010. – 640 p.

8. Luk′janchuk, V. D. Aktoprotektory: farmakologija ta farmakoterapija [Text] / V. D. Lu-k′janchuk, I. V. Simonova // Farmakologija ta likars'ka toksykologija. – 2015. – Issue 2. – P. 14–26.

9. Lesiovskaja, E. E. Metaprot pri jekstremal'nyh vozdejstvijah [Metaprot under extreme conditions] [Text] / E. E. Lesiovskaja. – Sankt-Peterburg: Polet, 2010. – 103 p.

10. Stepanjuk, G. I. Vplyv pohidnyh tetrazolo[1,5-s]hinazolinu na fizychnu vytryvalist' shhuriv pry riznyh temperaturnyh rezhymah [Effect of the tetrazol[1,5-c]quinazolin derivatives on the physical endurance of rats at different temperatures] [Text] / G. I. Stepanjuk, O. Ju. Tozjuk, N. G. Chornoivan, S. I. Kovalenko, O. Ju. Voskobojnik // Ukrai'ns'kyj zhurnal klinichnoi' ta laboratornoi' medycyny. – 2012. – Vol. 7, Issue 4. – P. 81–85.

11. Tozjuk, O. Ju. Harakterystyka emocijno-povedinkovyh reakcij shhuriv v umovah hronichnogo imobilizacijnogo stresu na tli dii' pohidnogo 5-R-tio-tetrazolo[1,5-s]hinazolinu [Characteristics of the emotional and behavioral reactions of rats in chronic imobilization stress during treatment with 5-R-thio-tetrazol[1,5-c]quinazoline] [Text] / О. Ю. Тозюк // Zaporozhskyj medycynskyj zhurnal. – 2013. – Issue 5 (80). – P. 57–59.

12. Jakovljeva, L. V. Eksperymental'ne vyvchennja novyh adaptogennyh zasobiv [Experimental study of new drugs adaptogenic] [Text]: metod. rek. / L. V. Jakovljeva, O. Ja. Mishhenko, Ju. B. Lar′janovs'ka et. al. – Kyiv, 2009. – 35 p.

13. Samojlov, N. N. Yzuchenye termoprotektornoj aktyvnosty fyzyologycheskyh sovmestymyyh antyoksydantov [Study termoprotektornoy activity physiologically compatible antioxidants] [Text] / N. N. Samojlov, Y. V. Yl'yna // Kubanskyj nauch. med. vestn. – 2006. – Issue 12. – P. 82–84.

14. Kruglikova, G. O. Glutationperoksydazna ta glutationreduktazna aktyvnist' pechinky pislja vvedennja selenitu natriju [Glutathione peroxidase and glutathione reductase activity of the liver after administration of sodium selenite] [Text] / G. O. Kruglikova, I. M. Shturman // Український біохімічний журнал. – 1976. – Vol. 48, Issue 2. – P. 223–228.

15. Lapach, S. N. Statisticheskie metody v mediko-biologicheskih issledovanijah s ispol'zovaniem Exel [Statistical methods in medical and biological research using Excel] [Text] / S. N. Lapach, A. V. Chubenko, P. N. Babich. – 2-nd ed. – Kyiv: Morion, 2001. – 408 p.

References

1. Sel'e, G.; E. M. Kreps (Ed.) (1982). Stress bez distressa [Stress without distress]. Moscow: Progress, 124.

2. Nikolaidis, M. G., Kyparos, A., Spanou, C., Paschalis, V., Theodorou, A. A., Vrabas, I. S. (2012). Redox biology of exercise: an integrative and comparative consideration of some overlooked issues. Journal of Experimental Biology, 215 (10), 1615–1625. doi: 10.1242/jeb.067470

3. Morales-Alamo, D., Calbet, J. A. L. (2013). Free radicals and sprint exercise in humans. Free Radical Research, 48 (1), 30–42. doi: 10.3109/10715762.2013.825043

4. Stankovic, M., Radovanovic, D. (2012). Oxidative stress and physical activity. Sportlogia, 8 (1), 1–11. doi: 10.5550/sgia.120801.en.001s

5. Ji, L. L., Zhang, Y. (2013). Antioxidant and anti-inflammatory effects of exercise: role of redox signaling. Free Radical Research, 48 (1), 3–11. doi: 10.3109/10715762.2013.844341

6. Pingitore, A., Lima, G. P. P., Mastorci, F., Quinones, A., Iervasi, G., Vassalle, C. (2015). Exercise and oxidative stress: Potential effects of antioxidant dietary strategies in sports. Nutrition, 31 (7-8), 916–922. doi: 10.1016/j.nut.2015.02.005

7. Olejnik, S. A., Guninoj, L. M., Sejfulla, R. D. (Eds.) (2010). Farmakologija sporta [Sports Pharmacology]. Kyiv: Olimpijskaja literatura, 640.

8. Luk′janchuk, V. D., Simonova, I. V. (2015). Aktoprotektory: farmakologija ta farmakoterapija. Farmakologija ta likars'ka toksykologija, 2, 14–26.

9. Lesiovskaja, E. E. (2010). Metaprot pri jekstremal'nyh vozdejstvijah [Metaprot under extreme conditions]. Sankt-Peterburg: Polet, 103.

10. Stepanjuk, G. I., Tozjuk, O. Ju., Chornoivan, N. G., Kovalenko, S. I., Voskobojnik, O. Ju. (2012). Vplyv pohidnyh tetrazolo[1,5-s]hinazolinu na fizychnu vytryvalist' shhuriv pry riznyh temperaturnyh rezhymah [Effect of the tetrazol[1,5-c]quinazolin derivatives on the physical endurance of rats at different temperatures]. Ukrai'ns'kyj zhurnal klinichnoi' ta laboratornoi' medycyny, 7 (4), 81–85.

11. Tozjuk, O. Ju. (2013). Harakterystyka emocijno-povedinkovyh reakcij shhuriv v umovah hronichnogo imobilizacijnogo stresu na tli dii' pohidnogo 5-R-tio-tetrazolo[1,5-s]hinazolinu [Characteristics of the emotional and behavioral reactions of rats in chronic imobilization stress during treatment with 5-R-thio-tetrazol[1,5-c]quinazoline]. Zaporozhskyj medycynskyj zhurnal, 5 (80), 57–59.

12. Jakovljeva, L. V., Mishhenko, O. Ja., Lar′janovs'ka, Ju. B. et. al. (2009). Eksperymental'ne vyvchennja novyh adaptogennyh zasobiv [Experimental study of new drugs adaptogenic]. Kyiv, 35.

13. Samojlov, N. N., Yl'yna, Y. V. (2006). Yzuchenye termoprotektornoj aktyvnosty fyzyologycheskyh sovmestymyyh antyoksydantov [Study termoprotektornoy activity physiologically compatible antioxidants]. Kubanskyj nauch. med. vestn., 12, 82–84.

14. Kruglikova, G. O., Shturman, I. M. (1976). Glutationperoksydazna ta glutationreduktazna aktyvnist' pechinky pislja vvedennja selenitu natriju [Glutathione peroxidase and glutathione reductase activity of the liver after administration of sodium selenite]. Ukrai'ns'kyj biohimichnyj zhurnal, 48 (2), 223–228.

15. Lapach, S. N., Chubenko, A. V., Babich, P. N. (2001). Statisticheskie metody v mediko-biologicheskih issledovanijah s ispol'zovaniem Exel [Statistical methods in medical and biological research using Excel]. Kyiv: Morion, 408.

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