Short Communication

Age Peculiarity Manifestation of Antioxidant Effect of 6-hexyl-3,6-dimethyl-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one on Rats under the Intermittent Hypobaric Hypoxia

Volodymyr Mykolayovuch Shvets[1], Alexei Yuriyevich Voskoboynik[2], Galina Grigorievna Berest[3], Sergiy Ivanovych Kovalenko[4], Maxim Vyacheslavovich Agibailov[5], Oleg Konstantynovuch Kulchitskiy[6], Nataliya Olegivna Kulchitska[7], Vadim Vyacheslavovich Davydov[8]

Departments of [1]Biochemistry, [2]Organic and Bioorganic Chemistry, [3]Pharmacognosy, Pharmacology and Medicine Technology, and [4]Organic and Bioorganic Chemistry, [5]Clinical Pharmacy, Pharmacotherapy, Management and Economics of Pharmacy, Zaporizkij Derzhavnij Medichnij Universitet, Zaporizhzhya, Ukraine, [6]Department of Biology of Aging, [7]Metabolism regulation laboratory, Institute of Gerontology, Kiev, Ukraine, [8]Department of Biochemistry and Molecular Biology, Rossijskij Nacional'nyj Issledovatel'skij Medicinskij Universitet Imeni N I Pirogova, Moscow, Russia

Address for correspondence:
Dr. Volodymyr Mykolayovuch Shvets, Department of Biochemistry, Zaporizkij Derzhavnij Medichnij Universitet, Zaporizhzhya 69001, Zaporiz'ka Oblast, Ukraine.
E-mail: v.n.shvets@gmail.com

Access this article online

Website: www.jrpsjournal.com

DOI:10.4103/jrptps.JRPTPS_30_19

Quick Response Code:

Abstract

Background: The organism of an old human as well as an experimental animal has decreased adaptive abilities and increased sensitivity to the effect of adverse environmental factors. We point out the high relevance of the search of new pharmacological compounds that have strong antioxidant properties and can be used to treatment and prevention of cardiovascular diseases with ageing. **Aims and Objective:** The aim of the study was to investigate the antioxidant activity of 6-hexyl-3,6-dimethyl-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (HTQ). **Materials and Methods:** Two groups of nonlinear male Wistar rats: 6–8 months (adult) and 24–26 months (old) were used. Determination of the myocardial diene conjugates and products reacting with 2-thiobarbituric acid (TBA-AP) concentration was carried out. **Results:** Concentrations of diene conjugates in adult and old rats, which were administered HTQ, were 21% and 32%, respectively, lower compared to animals to which the synthesized compound was not administered. After administration of HTQ, the concentration of TBA-AP in old animals decreased by 23% as compared with the animals of the control group. **Conclusion:** An administration of HTQ has limited the accumulation of intermediate lipid peroxidation products in the hearts of old animals in the condition of hypoxia.

Keywords: *Aging, hypoxia, lipid peroxidation, myocardium*

Introduction

It is a fact that the organism of an old human as well as an experimental animal has decreased adaptive abilities and increased sensitivity to the effect of adverse environmental factors.[1,2] In this case, the processes of free radical oxidation play an important role in the development of adaptive reactions. Changes in these processes are accompanied by the reduction in stability of the cardiovascular system against stress injuries. Although these injuries are related to stimulation of lipid peroxidation (LPO)[3,4] and a decrease in antioxidant activity of the cardiovascular system,[5,6] antioxidants are widely used nowadays. Vitamin E is one of the most common. According to the published data, it is a nontoxic,[7] but having some adverse effects compound. There is evidence that vitamin E may increase the risk of hemorrhagic stroke,[8,9] reduce the level of vitamin-K-dependent coagulation factors,[10-12] increase the congestive heart failure risk,[13,14] and even increase all-cause mortality when it is used in high doses.[15] Taking into account all aforementioned effects, we point out the high relevance of the search of new pharmacological compounds that have strong antioxidant properties and can be used to treatment and prevention of cardiovascular diseases with ageing. In this regard, the aim of this study was to investigate the effect of new synthetic antioxidants on LPO in the heart of rats of different ages under the intermittent hypobaric hypoxia. The aforementioned compound[16] is a low-toxic experimental drug that reveals high lipid lowering activity and increases the level of high-density lipoproteins.

Materials and Methods

Two groups of nonlinear male, Wistar rats: 6–8 months (adult) and 24–26 months (old), were used. Animals were kept in constant environmental conditions (temperature = 20 °C, circadian rhythm = 12L:12D) and were on a normal laboratory diet. Each group was divided into four subgroups: (1) intact (six rats); (2) control (were kept in the pressure chamber at a "height" of 5000 m for 7 h daily for six days,

How to cite this article: Shvets V, Voskoboynik A, Berest G, Kovalenko S, Agibailov M, Kulchitskiy O, *et al.* Age peculiarity manifestation of antioxidant effect of 6-hexyl-3,6-dimethyl-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one on rats under the intermittent hypobaric hypoxia. J Rep Pharm Sci 2019;8:295-7.

"lift" and "descent" rate—1000 m/h) (six rats); (3) group, which was orally administered 5 mg/100 g of 6-hexyl-3,6-dimethyl-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (HTQ) 60 min before being kept in the pressure chamber (six rats); and (4) group, which was orally administered vitamin E (alpha-tocopherol) at a dose of 33.5 mg/kg 60 min before being kept in the pressure chamber (six rats).[17]

The HTQ (compound) was synthesized at the Department of Organic and Bioorganic Chemistry at Zaporizhzhia State Medical University.[16,18]

The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals published in the United States by the National Institutes of Health.[19]

Twenty-four hours after the "descent" rats were decapitated. Hearts were immediately extracted. Left ventricles were immediately frozen in liquid nitrogen.

Frozen fragments of myocardium were homogenized with a mixture of heptane:isopropanol (1:1) to extract the diene conjugates (DCs).[20] The concentration of DCs was determined in a spectrophotometer at 232 nm by the method of Recknagel and Goshal[21] and expressed in nmol/g of myocardial tissue.

Some myocardial samples were homogenized with chilled 5% trichloroacetic acid. The homogenates were centrifuged at 3000 rpm for 10 min at 4°C. The concentration of products reacting with 2-thiobarbituric acid (TBA-AP) in protein-free supernatants was determined according to Muller.[22] The concentration of TBA-AP was expressed in nmol/g of the myocardium.

Statistical processing of the data was carried out using the nonparametric Wilcoxon–Mann–Whitney method.

Results

Study has shown that the effect of intermittent hypoxia on rats of both age groups was manifested by an increase of DC concentration in the heptane phase of lipid extract [Table 1]. It was more significant in old rats than in adult ones (56% and 30%, respectively, $P \leq 0.05$). The obtained data have indicated a more intensive stimulation of fatty-acid peroxidation in the hearts of old rats under hypoxia.

Concentrations of DCs in adult and old rats, which were administered HTQ, were 21% and 32%, respectively, lower compared to animals to which the synthesized compound was not administered. The obtained data have indicated that the effect of used compound on a stress stimulated free radical lipid oxidation was less significant in the case of adult rats than in the case of old ones.

The level of TBA-AP in the myocardium of both age group rats under the intermittent hypoxia increased as compared with intact group animals, more significant in old ones, than in adults (31% and 18%, respectively). Thus, content of lipid peroxidation products (TBA-AP) in myocardium of old intact rats remained at the same level as in adult ones. The obtained data have indicated the stable level process of free radical oxidation in myocardium of rats with different ages.

After administration of HTQ, the concentration of TBA-AP in old animals decreased by 23% as compared with the animals of the control group.

Figure 1 shows that administration of vitamin E supplement brings to decrease accumulation of DCs in adult and old rats by 24% and 39%, respectively, as compared with control group. Simultaneously the level of TBA-AP has decreased by 12% in adult and by 25% in old animals, respectively, compared to control group.

It was found that HTQ reveals antioxidant activity, which is comparable with effect of vitamin E.

Discussion

Estimating the results, we can see that HTQ has a noticeable antioxidant activity *in vivo*. The antioxidant effect of HTQ is manifested in the inhibition of LPO in the myocardium, stimulated by hypobaric hypoxia. This is confirmed by a decrease in the content of intermediate LPO products in the heart muscle. Therefore, we propose the revealing of the protective effect of HTQ on myocardium in the case of its ischemia and reperfusion.

Age dependence is characteristic for the manifestation of the antioxidant effect of HTQ, as well as for vitamin E. Age differences in the stimulation of LPO in myocardium under stress showed a special efficiency of the compound antioxidant properties on the heart with ageing. This is especially important

Table 1: Effect of 6-hexyl-3,6-dimethyl-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one on free radical lipid oxidation products concentration in the heart of rats of different age under the hypobaric hypoxia (M ± m)

Index	Adult (6–8 months, *n* = 24)				Old (24–26 months, *n* = 24)			
	Intact	Control	Exp. hypoxia + compound	Exp. hypoxia + vit. E	Intact	Control	Exp. hypoxia + compound	Exp. hypoxia + vit. E
DC (nmol/g of tissue)	26 ± 1.6	34 ± 1.7*	27 ± 1.9**	26 ± 1.8**	30 ± 1.6	47 ± 1.7*	32 ± 1.6**	29 ± 1.8**
TBA (nmol/g of tissue)	4.96 ± 0.36	5.86 ± 0.23*	5.74 ± 0.28	5.20 ± 0.31**	5.15 ± 0.33	6.76 ± 0.74*	5.20 ± 0.37**	5.10 ± 0.35**

*$P < 0.05$ as compared to an intact group
**$P < 0.05$ as compared to a control group

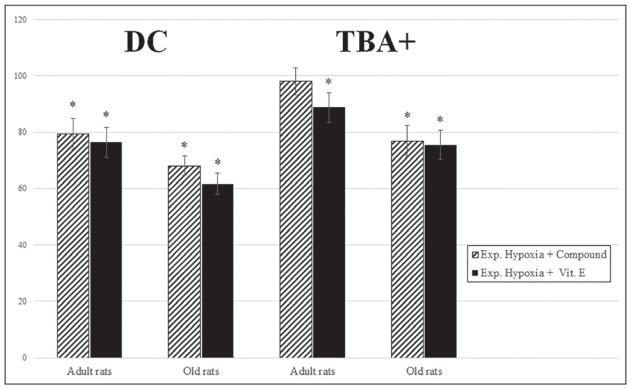

Figure 1: Antioxidant activity of 6-hexyl-3,6-dimethyl-6,7-dihydro-2H-[1,2,4] triazino[2,3-c]quinazolin-2-one and vitamin E. The ratio of LPO intermediates concentration in myocardium from control group rats were expressed as 100%. DC = conjugated dienes; TBA+ = TBA-positive substances. * *P* < 0.05 as compared to a control group

because of the decrease of myocardial antioxidant system activity and increase of the prooxidant factors effect on the myocardium occurs at senescence.[23-26]

The mechanism of antioxidant effect of HTQ on the heart is still not clear. By its nature and intensity, it is quite consistent with the effect of vitamin E. We can assume that the basis of its antioxidant effect is the property to act as a trap for free radicals.

There are not known causes of age-related features in revealing of the action of this compound on the myocardium. The causes of age-related features in the manifestation of the synthesized compound effect on myocardium are not explored too. Obviously, they can be associated with age differences in the level of basal activity of myocardial antioxidant system. Perhaps they are associated with a different availability of compound in different age groups of animals. Our further research will be devoted to the study of this issue.

Conclusion

It was found that HTQ reveals antioxidant activity, which is comparable with effect of vitamin E, and significantly decreases the concentration of DCs and TBA-AP in myocardium of old (24–26 months) Wistar rats under the intermittent hypobaric hypoxia. Effect of the compound is characterized by age dependence and it is more pronounced in old animals.

References

1. Frolkis VV, Bezrykov VV, Kulchitsky OK. Aging and experimental age pathology of the cardiovascular system. Naukova Dumka 1994;248:18-21.
2. Kulchitsky OK, Potapenko RI, Novikova SN, Shvets VN. Change of nitrogen oxide under intermittent hypoxia in rats of different age. Circ Hemostasis 2013;3–4:138-46.
3. Meerson FZ. Pathogenesis and prevention of heart's stress injure. Moscow: Medicine; 1984.
4. Davydov VV, Shvets VN. Lipid peroxidation in the heart of adult and old rats during immobilization stress. Exp Gerontol 2001;36:1155-60.
5. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008;4:89-96.
6. McIntyre TM, Hazen SL. Lipid oxidation and cardiovascular disease: Introduction to a review series. Circ Res 2010;107:1167-9.
7. Bendich A, Machlin LJ. Safety of oral intake of vitamin E. Am J Clin Nutr 1988;48:612-9.
8. Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. NEJM 1994;15:1029-35.
9. Schürks M, Glynn RJ, Rist PM, Tzourio C, Kurth T. Effects of vitamin E on stroke subtypes: Meta-analysis of randomised controlled trials. BMJ 2010;341:c5702.
10. Corrigan JJ Jr, Marcus FI. Coagulopathy associated with vitamin E ingestion. JAMA 1974;230:1300-1.
11. No authors listed. Vitamin K, vitamin E and the coumarin drugs. Nutr Rev 1982;6:180-2.
12. Meyers DG, Maloley PA, Weeks D. Safety of antioxidant vitamins. Arch Intern Med 1996;156:925-35.
13. Lonn E, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, *et al.*; HOPE and HOPE-TOO Trial Investigators. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA 2005;293:1338-47.
14. Marchioli R, Levantesi G, Macchia A, Marfisi RM, Nicolosi GL, Tavazzi L, *et al.*; GISSI-Prevenzione Investigators. Vitamin E increases the risk of developing heart failure after myocardial infarction: results from the GISSI-prevenzione trial. J Cardiovasc Med (Hagerstown) 2006;7:347-50.
15. Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: High-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 2005;142:37-46.
16. Voskoboynik OY, Kolomoets OS, Antypenko OM, Zhernova GO, Nosulenko IS, Berest GG, *et al*. Synthesis and hypolipidemic activity of new 6,6-disubstituted 3-R-6,7-dihydro-2H-[1,2,4]triazino[2,3-c] quinazolin-2-ones. J Heterocyclic Chem 2018;55:318-25.
17. Asha Devi S, Manjula KR, Subramanyam MV. Protective role of vitamins E and C against oxidative stress caused by intermittent cold exposure in aging rat's frontoparietal cortex. Neurosci Lett 2012;529:155-60.
18. Voskoboinik OY, Kolomoiets OS, Kovalenko SI, Berest HH, Kholodniak SV, Serheieva TY, *et al*. inventors; Zaporizhia state medical university, assignee. 6-Mono- and 6,6-disubstituted 3-R-8-R3-9-R4-10-R5-11-R6-6,7-dihydro-2H-[1,2,4]triazino[2,3-c] quinazolin-2-ones. Ukraine patent 111245. April 11, 2016.
19. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. 8th ed. Washington: The National Academies Press; 2011.
20. Kates M. Techniques of lipidology: isolation, analysis and identification of lipids. Amsterdam: Elsevier; 1972. p. 356-8.
21. Recknagel RO, Ghoshal AK. Quantitative estimation of peroxidative degeneration of rat liver microsomal and mitochondrial lipids after carbon tetrachloride poisoning. Exp Mol Pathol 1966;5:413-26.
22. Müller G, Frühauf A, Mathias B. [Thiobarbituric acid positive substances as indicators of lipid peroxidation]. Z Gesamte Inn Med 1986;41:673-6.
23. Venkataraman K, Khurana S, Tai TC. Oxidative stress in aging—matters of the heart and mind. Int J Mol Sci 2013;14:17897-925.
24. Karavidas A, Lazaros G, Tsiachris D, Pyrgakis V. Aging and the cardiovascular system. Hellenic J Cardiol 2010;51:421-7.
25. Dai DF, Rabinovitch PS, Ungvari Z. Mitochondria and cardiovascular aging. Circ Res 2012;110:1109-24.
26. Wu J, Xia S, Kalionis B, Wan W, Sun T. The role of oxidative stress and inflammation in cardiovascular aging. Biomed Res Int 2014;2014:615312.