ENALAPRIL EFFECT ON GLUTATHIONE CHAIN OF THE ANTIOXIDANT SYSTEM OF THE BRAIN IN RATS WITH SCOPOLAMINE-INDUCED NEURODEGENERATION

Kmet O., Filipets N., Kmet T., Hrachova T., Vepriuk Y.

Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine

Current research programs are directed to elaboration of strategies of effective prognosis, prevention and treatment of functional disorders of the central nervous system. It especially refers to dementia, in particular those developing with Alzheimer's disease, Hallervorden-Spatz disease, Parkinson's disease, since their pathogenic mechanisms are associated with neurodegenerative processes [8, 16]. The most widely spread sign for the majority of neurodegenerative disorders is an excessive formation of oxygen reactive forms due to oxidative stress, causing damage and loss of neuronal cells [9]. Under conditions of neurodestruction progress the activity of the antioxidant system decreases and formation of free radicals increases, inducing damage of tissues, changing oxidation-reduction state of cells followed by further activation of redox-sensitive genes. Therefore, oxidative stress is a cause and a leading component of many pathological processes of the central nervous system and neurodegenerative diseases in particular [14, 21].

The brain is the most sensitive to oxidative damage [13]. Glutathione system plays an important role in realization of anti-radical protection of neurons. Coordinated action of all its components (reduced glutathione, glutathione peroxidase, glutathione reductase, sulfhydryl groups) promotes restoration of an optimal level of peroxide compounds and maintenance of the pro-antioxidant balance [1, 5]. Moreover, glucose-6-phosphate dehydrogenase, possessing a key position in glutathione metabolism, is a source of pentose essential for the synthesis and reparation of DNA. Thus, the degree of glutathione system damage is closely connected with cytotoxic effects. At the same time, disorders in the glutathione system take a leading role in realization of neurodegeneration mechanisms caused by oxidative stress [4].

Results of experimental and clinical studies in recent years enable to consider that activation of renin-angiotensin system (RAS) of the brain is one of pathogenic chains of oxidative damage of the neuron cellular membranes due to increased generation of oxygen reactive forms [6, 22]. Considering the fact that due to establishment of non-cardiovascular effects of RAS, and availability of the central neurotropic effects in particular (ability to reduce oxidative stress and apoptosis), the possibilities of pharmacological blockers of RAS under conditions of development of neurodegenerative changes in the brain neurons are of a special interest.

Objective of the work is to study enalapril effect, a blocker of renin-angiotensin system, on glutathione chain of the antioxidant system of the cerebral cortex and hippocampus of rats with experimental neurodegeneration.

Material and methods. The experiments were conducted on nonlinear albino male rats 0,18-0,20 kg of the body weight, kept under standard vivarium conditions at the temperature of 18-22 °C and relative humidity 40-60 %, fed on balanced food allowance and free access to water. All the experiments with animals were conducted according the main principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986). All the rats were randomized into two groups: 1 – control group; 2 – group with neurodegeneration model. Considering the common recognition of cholinergic hypothesis in pathogenesis of neurodegenerative changes in the central nervous system the experiments were conducted under conditions of scopolamine-induced damage of the brain [12]. To create the model scopolamine hydrochloride (Sigma, USA) was injected intraperitoneally (i/p) in the dose of 1 mg/kg of the body weight in the form of 0,01 % water solution, once a day during 27 days. The rats from the control group received physiological solution only in the analogical regimen and experimental conditions.

On the 28th day the rats with modeled pathology were randomized into two groups: I – i/p administration of enalapril (Zdorovye, Ukraine) in the dose of 1 mg/kg and II – 1 ml of saline only during 14 days [18]. The control rats received 1 ml of saline since the 28th day. Euthanasia of the animals was performed under light ether narcosis. At a cold temperature the brain was removed, carefully washed with cool 0,9 % NaCl solution, and the cerebral cortex and hippocampus were isolated according to the coordinates of the stereotaxic atlas [19], since these parts of the brain are the first to suffer in case of neurodegenerative processes [3, 20]. Cytoplasmic fraction was isolated by means of the method of differentiation centrifuging of homogenate of the cerebral cortex and hippocampus on the refrigerator centrifuge at 1000 g 10 min, then 1400 g 10 min at a temperature of 4 °C. To assess the state of the antioxidant system of the cerebral cortex and hippocampus the contents of sulfhydryl (SH-) groups [10], reduced glutathione (G-SH) and activity of glutathione-reductase (GR) [EC 1.6.4.2], glutathione-peroxidase (GP) [EC 1.11.1.9], glucose-6-phosphate dehydrogenase (G-6-PDH) [EC 1.1.1.49] were determined by means of certain methods [15]. Lowry protein assay determined the amount of protein in specimens [7].

The results of the study were statistically processed by means of Student t-criterion. Distribution of values in samples was preliminary checked in order to prove an adequate method of statistical assessment of a mean difference between the groups of the study. According to Shapiro-Wilk criterion the data concerning distribution deviation in samples from that of the norm were not obtained ($p>0,05$). Taking into account the above-mentioned application of Student t-criterion was considered to be sufficient to obtain valid conclusions. At the same time, to prove reliability of conclusions Mann-Whitney non-parametric comparison criterion was applied, which showed similar results of calculations by means of Student t-criterion concerning p value. Therefore, $p\leq0,05$ was considered to be a sufficient level of discrepancy probability.

Results and their discussion. Glutathione system takes a leading role in maintenance of SH - S-S exchange in the tissues at the expense of transformation of reduced glutathione form into oxidized one essential for performance of such vital processes in the cells as functioning of membranous structures, cellular skeleton and cellular division. Exhaustion of functional possibilities of glutathione system results in activation of free radical oxidation, increased permeability of the cellular membranes for Ca^{2+} ions, activation of phospholipase and endonuclease, which in its turn is a cause of free radical or enzymatic damage of DNA molecules [2].

Table. Enalapril effect on the indices of glutathione system in cytosolic fraction of rats with Scopolamine-induced neurodegeneration (M±m, n=7)

Indices	Brain structures	Control	Neurodegeneration model	Neurodegeneration model + Enalapril
Reduced glutathione (mcmol/(g of tissue))	Cerebral cortex	7,373±0,600	2,698±0,339*	4,942±0,283*,**
	Hippocampus	6,839±1,018	4,247±0,589*	5,183±0,784**
Glutathione peroxidase (nmol ГSSГ/(min of mg of protein))	Cerebral cortex	143,174±13,988	99,594±7,250*	121,880±10,545
	Hippocampus	131,460±15,549	88,277±10,931*	110,519±10,066
Glutathione reductase (nmol NADPH /(min of mg of protein)	Cerebral cortex	3,710±0,486	1,995±0,404*	3,475±0,270**
	Hippocampus	3,464±0,461	2,062±0,441*	3,317±0,215**
Glucose-6-phosphate dehydrogenase (nmol/(min of mg of protein)	Cerebral cortex	6,286±0,110	4,938±0,481*	5,199±0,677
	Hippocampus	4,834±0,366	3,475±0,495*	3,594±0,274*
Sulfhydryl groups (nmol/(min of mg of protein)	Cerebral cortex	72,813±2,357	50,548±2,907*	63,945±3,613**
	Hippocampus	70,575±3,795	54,834±3,101*	59,276±1,354**

*notes: * – significant difference compared to that of the control group;*
*** – significant difference compared to that of the neurodegeneration model*

The present study investigates the dynamics of G-SH changes in the cerebral cortex and hippocampus (Table). Thus, in comparison with the control group, the content of G-SH in rats with scopolamine-induced neurodegeneration 63,4 and 36,6 % decreased in the cerebral cortex and hippocampus respectively. Such differences are most likely caused by increased use of G-SH for inactivation of excessive amount of free radicals in the damaged neurons of the examined cerebral structures and inhibition of the process of G-SH regeneration from the oxidized form. At the same time, a decreased activity of the enzyme of NADPH-dependent GR participating in the process of antioxidant protection is found – 46,4 % in the cerebral cortex and 40,5 % - in the hippocampus.

In rats with neurodegeneration GP activity, which uses G-SH for neutralization of hydrogen peroxide and other hydroxyperoxides, was lower than that of the control group: 30,4 % – in the cerebral cortex; and 32, 9 % – in the hippocampus. The content of SH-groups contained in glutathione and providing biochemical reactions of metabolism and maintenance of membranous functions decreased as well: 30,6 % in the cerebral cortex and 22,3 % – in the hippocampus.

One of the ways to adapt the metabolism of cerebral tissues to hypoxic conditions is activation of pentose phosphate way of oxidation. Therefore, we have examined G-6-PDH activity in the examined cerebral structures of rats. Decreased activity of G-6-PDH was found to be 21,6 and 27,9 % in the cerebral cortex and hippocampus respectively in rats with scopolamine-induced neurodegeneration in comparison with the indices in the control group.

Further analysis demonstrated that after administration of enalapril the indices of antioxidant protection in the brain increased in rats with neurodegeneration. Comparison of the data obtained in the modeled pathology with the indices of rats subjected to enalapril determined increased content of G-SH in the cerebral cortex 1,8 times as much and in the hippocampus – 1,2 times. Under enalapril effect the content of SH-groups in the cerebral cortex and hippocampus increased 1,3 and 1,1 times respectively. Increasing of G-SH content is likely to occur at the expense of its intensified regeneration from the oxidized form in the tissues of the cerebral cortex and hippocampus. A positive effect of enalapril was characterized by an increased activity of GR in the cerebral cortex 1,7 times as much, and 1,6 times – in the hippocampus.

In view of the absence of significant differences in the activity of G-6-PDH in the enalapril group compared to the pathology, the cause of the detected changes in the investigated structures can be considered to be the involvement of this enzyme in the pentosephosphate pathway of carbohydrate metabolism to stabilize oxidation-reduction processes in the brain. One of the functions of pentose phosphate way of carbohydrate metabolism is supply of NADPH reduced equivalents essential for energy production and restoration of oxidized glutathione in the brain.

Therefore, the conducted experimental studies determined that enalapril increases activity of the brain antioxidant system under conditions of development of Scopolamine-induced neurodegeneration in rats. Increase of the antioxidant protection is first of all caused by angiotensin II (AII) inhibition – the main RAS effector. In addition to powerful vasoconstriction it stimulates NADPH-oxidase, which excessive production plays a key role in the development of oxidative and inflammatory processes, intensifies neuron susceptibility and induces neuronal mitochondrial dysfunction [17]. A positive effect of enalapril can be explained by the fact of formation of other kinds of angiotensin with decreased activity of AII formation - A-1-7, AIII, AIV in particular [11]. These peptides cause additional stimulation of appropriate receptors, and thus promote additional vasodilation, anti-proliferation action and regeneration of tissues. At the same time, cerebral circulation improves due to systemic vasoconstriction effects of AII, and the processes of antioxidant protection, essential for cerebral functioning, intensify.

Conclusions.

1.Scopolamine-induced neurodegeneration reduces the content of reduced glutathione, sulfhydryl groups, activity of glutathione reductase, glutathione peroxidase and glucose-6-phosphate

dehydrogenase in the cerebral cortex and hippocampus, which is indicative of inhibition of the antioxidant protection system.
2.Administration of enalapril, a blocker of renin-angiotensin system, increases the content of reduced glutathione and sulfhydryl-groups, activity of glutathione-dependent enzyme – glutathione reductase in the cerebral cortex and hippocampus of rats with scopolamine-induced neurodegeneration.
3.Improvement of the antioxidant protection glutathione chain in the cerebral cortex and hippocampus is indicative of enalapril ability to inhibit pathogenic mechanisms of neurodegenerative processes caused by tissue renin-angiotensin system in the central nervous system.

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SUMMARY

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Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», Chernivts, Ukraine

Objective of the work is to study enalapril effect, a blocker of renin-angiotensin system, on glutathione chain of the antioxidant system of the cerebral cortex and hippocampus of rats with experimental neurodegeneration.

The experiments were conducted on nonlinear laboratory albino male rats with their body weight of 0,18-0,20 kg. The model of neurodegeneration was created by means of intraperitoneally administration of scopolamine hydrochloride (Sigma, USA) during 27 days in the dose of 1 mg/kg. Since the 28th day of the experiment enalapril (Zdorovye, Ukraine) was introduced intraperitoneally in the dose of 1 mg/kg in 1 ml of physiological solution once a day during 14 days.

The content of reduced glutathione in male rats with scopolamine-induced neurodegeneration after introduction of enalapril increased in the cerebral cortex 1,8 times as much, and in the hippocampus – 1,2 times. Under enalapril effect the content of sulfhydryl groups increased in the cerebral cortex and hippocampus 1,3 and 1,1 times respectively. A positive effect of enalapril was characterized by an increased activity of glutathione

reductase in the cerebral cortex 1,7 times as much, and 1,6 times – in the hippocampus.

Thus, enalapril improves the indices of glutathione chain of the antioxidant system of the cerebral cortex and hippocampus, which is indicative of its neuroprotective ability under conditions of scopolamine-induced damage and development of neurodegenerative processes in rats.

Keywords: scopolamine-induced neurodegeneration, brain, enalapril, glutathione chain.

РЕЗЮМЕ

ВЛИЯНИЕ ЭНАЛАПРИЛА НА ГЛУТАТИОНОВОЮ ЦЕПЬ АНТИОКСИДАНТНОЙ СИСТЕМЫ МОЗГА КРЫС СО СКОПОЛАМИН-ИНДУЦИРОВАННОЙ НЕЙРОДЕГЕНЕРАЦИЕЙ

Кметь О.Г., Филипец Н.Д., Кметь Т.И., Грачева Т.И., Вепрюк Ю.М.

Высшее государственное учебное заведение Украины «Буковинский государственный медицинский университет», Черновцы, Украина

Цель исследования - изучить влияние блокатора ренин-ангиотензиновой системы эналаприла на глутатионовою цепь антиоксидантной системы коры головного мозга и гиппокампа крыс с экспериментальной нейродегенерацией.

Эксперименты проводили на нелинейных лабораторных белых крысах самцах массой 0,18-0,20 кг. Модель нейродегенерации создавали внутрибрюшинным введением в течение 27 дней скополамина гидрохлорида (Sigma, США) в дозе 1 мг/кг. Начиная с 28 суток эксперимента, эналаприл вводили внутрибрюшинно в дозе 1 мг/кг в 1 мл физиологического раствора - один раз в день в течение 14 дней.

У крыс самцов со скополамин-индуцированной нейродегенерацией после введения эналаприла в коре головного мозга увеличивалось содержание глутатиона восстановленного в 1,8 раза и в гиппокампе - в 1,2 раза. Под влиянием эналаприла содержание сульфгидрильных групп повышалось в коре головного мозга и гиппокампе в 1,3 и 1,1 раза, соответственно. Положительное влияние эналаприла характеризовалось повышением активности глутатион-редуктазы в коре в 1,7 раза и в гиппокампе - в 1,6 раза.

Таким образом, эналаприл улучшает показатели глутатионовой цепи антиоксидантной системы коры головного мозга и гиппокампа, что указывает на его нейропротекторную способность в условиях скополамин-индуцированного повреждения и развития нейродегенеративных процессов у крыс.

რეზიუმე

ენალაპრილის გავლენა სკოპოლამინ-ინდუცირებული ნეიროდეგენერაციის მქონე ვირთაგვების ტვინის ანტიოქსიდაციური სისტემის გლუტათიონურ ჯაჭვზე

ო.კმეტი, ნ.ფილიპეცი, ტ.კმეტი, ტ.გრაჩოვა, ი.ვეპრიუკი

ბუკოვინის სახელმწიფო სამედიცინო უნივერსიტეტი, ჩერნოვცი, უკრაინა

კვლევის მიზანს წარმოადგენდა რენინ-ანგიოტენზინის სისტემის ბლოკატორის – ენალაპრილის გავლენის შეფასება თავის ტვინის ქერქის და ჰიპოკამპის ანტიოქსიდაციური სისტემის გლუტათიონურ ჯაჭვზე ვირთაგვებში ექსპერიმენტული ნეიროდეგენერაციით.

ექსპერიმენტები ჩატარდა 0,18-0,20 კგ წონის ლაბორატორიულ არახაზოვან მამრ თეთრ ვირთაგვებზე. ნეიროდეგენერაციის მოდელი იქმნებოდა 27 დღის განმავლობაში ინტრაპერიტონეულად სკოპოლამინის ჰიდროქლორიდის შეყვანით (Sigma, США), დოზით 1 მგ/კგ. ექსპერიმენტის 28-ე დღიდან 14 დღის განმავლობაში, დღეში ერთხელ, ინტრაპერიტონეულად შეიყვანებოდა ენალაპრილი, დოზით 1 მგ/კგ 1 მლ ფიზიოლოგიურ ხსნარში.

მამრ ვირთაგვებში სკოპოლამინ-ინდუცირებული ნეიროდეგენერაციით ენალაპრილის შეყვანის შემდეგ თავის ტვინის ქერქში გლუტათიონის შემცველობა იზრდებოდა 1,8-ჯერ, ჰიპოკამპში – 1,2-ჯერ. ენალაპრილის გავლენით სულფჰიდრილური ჯგუფების შემცველობა თავის ტვინის ქერქსა და ჰიპოკამპში იზრდებოდა, შესაბამისად, 1,3-ჯერ და 1,1-ჯერ. ენალაპრილის დადებითი გავლენა ხასიათდებოდა გლუტათიონ-რედუქტაზას აქტივობის გაზრდით თავის ტვინის ქერქში 1,7-ჯერ, ჰიპოკამპში – 1,6-ჯერ.

ამრიგად, ენალაპრილი აუმჯობესებს თავის ტვინის ქერქის და ჰიპოკამპის ანტიოქსიდაციური სისტემის გლუტათიონური ჯაჭვის მაჩვენებლებს, რაც მიუთითებს მის ნეიროპროტექტორულ შეასძლებლობაზე სკოპოლამინ-ინდუცირებული დაზიანებების და ნეიროდეგენერაციული პროცესების განვითარების პირობებში ვირთაგვებში.