ANALYSIS

24(106), November - December, 2020

ISSN
2321–7359
EISSN
2321–7367

Medical Science

New tendencies of proteolysis/fibrinolysis pharmacological modulation with experimental Alzheimer's disease

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Article History
Received: 15 September 2020
Reviewed & Revised: 17/September/2020 *to* 15/October/2020
Accepted: 15 October 2020
E-publication: 23 October 2020
P-Publication: November - December 2020

Citation
Olga Kmet, Nataliia Filipets, Taras Kmet, Yurii Vepriuk, Kateryna Vlasova. New tendencies of proteolysis/fibrinolysis pharmacological modulation with experimental Alzheimer's disease. *Medical Science*, 2020, 24(106), 3911-3917

ABSTRACT

Nowadays effective methods to prevent and treat Alzheimer's disease are lacking, therefore exploration of new tendencies concerning pathogenic therapy of the disease remains of topical interest. The fact that evaluation of biochemical condition of the damaged organs and systems assumes investigation of proteolysis/fibrinolysis markers deserves attention, since changes of the markers are considered as pathophysiological basis of many diseases and as a target of protective therapy. Therefore, objective of our study was to examine the effect of carbacetam, GABA-receptors modulator, and enalapril, angiotensin-converting enzyme inhibitor, on the proteolytic and fibrinolytic activity of the cerebral cortex and hippocampus of rats with Scopolamine-induced Alzheimer's disease. The experiments were conducted on nonlinear laboratory albino male rats with their body weight of 0.18-0.20 kg. Alzheimer's disease was simulated by administration of scopolamine hydrochloride (Sigma, USA) at a dose of 1 mg/kg for 27 days. Carbacetam and enalapril were administered intraperitoneally at a dose of 5 mg/kg and 1 mg/kg, once daily for 14 days. The indices of proteo- and fibrinolytic activity were determined in the homogenates of the cerebral cortex and hippocampus. Under conditions of Scopolamine-induced Alzheimer's disease proteolytic and fibrinolytic activity of the cerebral cortex and hippocampus of rats was found to increase. After carbacetam was administered to rats with Alzheimer's disease during 14 days, collagenolysis and enzymatic fibrinolytic activity in the cerebral cortex decreased, and low molecular proteinolysis – in the hippocampus only. Under enalapril effect proteolysis/fibrinolysis indices decrease in both structures of the brain examined. The results obtained confirm participation of the proteolytic and fibrinolytic systems in the mechanisms of neurodegeneration, and are indicative of reasonability to initiate pathogenic correction by means of the modulators of renin-angiotensin and GABA-systems under conditions of Alzheimer's disease development.

Keywords: enalapril, carbacetam, proteolysis, fibrinolysis, Alzheimer's disease

1. INTRODUCTION

Neurodegenerative processes are peculiar for a group of various diseases with underlying process promoting death of the neurons followed by further reduction of cognitive functions and development of dementia. Nowadays Alzheimer's disease is the most common cause of dementia. At the same time, there are no effective methods of prevention and treatment of Alzheimer's disease, therefore exploration of new tendencies concerning pathogenic therapy of the disease remains of topical interest. The fact that evaluation of biochemical condition of the damaged organs and systems assumes investigation of proteolysis/fibrinolysis markers deserves attention, since changes of the markers are considered as pathophysiological basis of many diseases and as a target of protective therapy (Gozhenko et al., 2017).

According to the latest scientific data plasmin proteolytic cascade is known to be concerned with formation of β-amyloid peptide – a pathogen of Alzheimer's disease able to aggregate into fibrils and be deposited in the form of extracellular plaques in the brain parenchyma (Klohs, 2019; Guo-fang Chen et al., 2017). This process begins with a localized synthesis and secretion of the tissue plasminogen activator (tPA) and urokinase plasminogen activator. In the processes of fibrinolysis tPA is conjugated with fibrin aggregates resulting in tPA conformational modification. It sharply increases its relationship to plasminogen and promotes transformation into active plasmin. Recent studies demonstrated that tPA is activated by β-amyloid in case of Alzheimer's disease (Constantinescu et al., 2017).

Increased fibrinogen level was found to result in changes of rheological properties of the blood, reactivity of the vessels and disturbance of the endothelial layer integrity (Petersen et al., 2018). Fibrinogen sedimentation with transformation into fibrin increases inflammation and permeability of vessels in the place of formation (Davalos and Akassoglou, 2012). Fibrin deposits in the central nervous system through the damaged hematoencephalic barrier enhance inflammation due to microglia activation (Iadecola, 2013; Sweeney et al., 2018). Therefore, fibrinogen increase promotes vascular pathology and neuron dysfunction. According to the latest data (Petersen et al., 2018), pharmacological decrease of fibrinogen level results in reduction of nervous-vascular pathology and inflammatory reaction in mice. On the contrary, when fibrinogen deposits are exhausted, occurrence of cerebral amyloid angiopathy and degree of cognitive disorders decrease.

Numerous scientific data are indicative of the fact that chronic inflammation is the main process promoting neurodegeneration advance. At the same time, a sufficient level of gamma-aminobutyric acid (GABA) in the neurons can decrease inflammation, and on the contrary, its deficiency is a cause of neuron loss (Błaszczyk, 2016; Bhandage et al., 2018; Cawley et al., 2015). GABA inhibition leads to relaxation of the smooth muscles of the cerebral vessels, increased permeability of the hematoencephalic barrier, development of inflammation of the brain vessels and tissue, tPA increase, thus – activation of neurodegeneration mechanisms (Labandeira-Garcia et al., 2017). Today pharmacological inhibition of angiotensin converting enzyme (ACE) is known to produce a

positive effect on the endogenous fibrinolytic balance due to inhibition of plasminogen-1, endothelin-1 and nitrogen oxide activator (Tetsuya Matsumoto and Minoru Horie, 2011). At the same time, renin-angiotensin system blockers promote bradykinin destruction and decelerate the cascade of neurodegenerative processes (Ahmed et al., 2018; Labandeira-Garcia et al., 2017). Since prolonged increase of pro-inflammatory cytokines content in the brain is a key factor promoting neuron degeneration including Alzheimer's disease, administration of ACE inhibitors for preventive and therapeutic purpose is reasonable (Takane et al., 2017).

Considering the above mentioned, in order to expand notion concerning the mechanisms of neuroprotection, investigation of proteolysis/fibrinolysis transformations in the brain of rats with Alzheimer's disease after administration of modulators of GABA and renin-angiotensin systems is considered to be reasonable. Objective of our study was to examine the effect of carbacetam, GABA-receptors modulator, and enalapril, angiotensin-converting enzyme inhibitor, on the proteolytic and fibrinolytic activity of the cerebral cortex and hippocampus of rats with Scopolamine-induced Alzheimer's disease.

2. MATERIAL AND METHODS

The experiments were conducted on nonlinear laboratory albino male rats with their body weight of 0.18-0.20 kg, kept under standard vivarium conditions with natural alternation of day and night. All the manipulations were conducted according to the Requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. Inaddition, they were confirmed by the Board on Biomedical Ethics Issues at the Higher State Educational Establishment of Ukraine «Bukovinian State Medical University» (Experimental Study, Reference № 59-20).

The model of Alzheimer's disease was created by means of intraperitoneal administration of Scopolamine hydrochloride (Sigma, USA) in the dose of 1 mg/kg once a day during 27 days (Kmet et al., 2019). The rats with Alzheimer's disease began course (14 days) administration of enalapril and carbacetam intraperitoneally at doses of 1 and 5 mg/kg body weight, respectively. Comparison groups: control (healthy) and rats with model pathology in a similar mode intraperitoneally injected solvent (0.9% NaCl solution). Euthanasia of rats was performed by blood letting under light ether anesthesia. The brain was removed cool and carefully washed with cool 0.9 % NaCl solution. The hippocampus and cerebral cortex were removed according to the stereotaxic (Paxinos and Watson, 2013). Brain homogenates were prepared in 0.05 M tris-HCl buffer (pH 7.4).

Proteolysis/fibrinolysis was investigated according to the method (Filipets, 2014) using the set of reagents Simko Ltd (Lviv, Ukraine). The state of proteolytic activity was determined on the basis of reaction with azoalbumin (low molecular proteinolysis (LMP)), azocasein (high molecular proteinolysis (HMP)) and azocollagen (collagenolysis). Fibrinolytic activity was examined evaluating the degree of homogenate staining due to plasmin formation in the presence of ε-aminocapronic acid (nonenzymatic fibrinolytic activity (NFA)) or without it (total fibrinolytic activity (TFA)). Enzymatic fibrinolytic activity (EFA) was determined by the difference between TFA and NFA. Proteolysis and fibrinolysis were estimated in E440/hour/mg of tissue, where E440 – extinction index for activity.

The results of the study were statistically processed applying parametric Student t-criterion. In case normal distribution was lacking Mann-Whitney U-criterion was applied. The differences were considered reliable with $p<0.05$. Point estimate of the results was represented in the form of mean values and standard mean error (M±m).

3. RESULTS

The study conducted demonstrated that in rats with Scopolamine-induced Alzheimer's disease proteolytic and fibrinolytic activity increased, and the indices changed after administration of both enalapril and carbacetam (Table 1 and 2). The state of proteolysis of the cerebral cortex was characterized by 22.5% increase of LMP lysis, 20.9% increase of HMP lysis, and collagenolysis – 53.8%. Similar dynamics of the indices occurred in the hippocampus. Thus, proteolysis by azoalbumin 17.7% increased, by azocasein – 16.3%, by azocollagen – 51.4%. Fibrinolysis in rats with Alzheimer's disease differed from that of the control by an average 13.4% increased values of TFA, NFA, EFA in the cerebral cortex, and in the hippocampus – 35.3; 42.5; 22.6% respectively.

Administration of carbacetam, GABA-receptors modulator, to rats with Alzheimer's disease promoted 14.8% decrease of collagenolysis in the cerebral cortex, and 5.2% decrease of LMP lysis in the hippocampus. After carbacetam administration fibrinolytic activity of the both examined structures of rats with Alzheimer's disease did not change, except EFA of the cerebral cortex, which index was 9.2% lower than that of the group not treated. Under effect of enalapril, angiotensin-converting enzyme inhibitor, proteolytic activity of the examined cerebral structures decreased. In comparison with the simulated pathology after administration of the drug LMP lysis in the cerebral cortex 7.1% decreased, in the hippocampus – 8.7%. HMP lysis in the cerebral cortex 8.1% decreased, in the hippocampus – 10.1%. Collagenolysis 16.9% decreased in the hippocampus only.

Table 1 Carbacetam and enalapril effect on proteolytic and fibrinolytic activity of the cerebral cortex of rats with Scopolamine-induced Alzheimer's disease (M±m)

Indices	Control	Alzheimer's disease	Alzheimer's disease + carbacetam	Alzheimer's disease + enalapril
Lysis azoalbumin, E440/(h×mg of tissue)	102.46±1.47	120.54±1.42*	117.23±2.13*	112.06±1.42* **
Lysis azocasein, E440/(h×mg of tissue)	104.37±1.19	121.37±2.26*	118.51±2.25*	111.60±0.89* **
Lysis azocollagen, E440/(h×mg of tissue)	2.49±0.14	3.77±0.19*	3.21±0.14* **	3.29±0.17*
TFA, E440/(h×mg of tissue)	86.11±1.43	97.43±2.05*	94.46±0.83*	88.97±1.33* **
NFA, E440/(h×mg of tissue)	57.34±1.04	65.29±1.90*	64.76±0.44*	60.43±0.51* **
EFA, E440/(h×mg of tissue)	28.77±0.77	32.71±0.60*	29.70±0.77* **	28.54±1.62* **

Note. * – reliability of differences compared with the control group of rats;
** – reliability of differences compared with the group of rats with Alzheimer's disease.

Table 2 Carbacetam and enalapril effect on proteolytic and fibrinolytic activity of the hippocampus of rats with Scopolamine-induced Alzheimer's disease (M±m)

Indices	Control	Alzheimer's disease	Alzheimer's disease + carbacetam	Alzheimer's disease + enalapril
Lysis azoalbumin, E440/(h×mg of tissue)	81.49±1.17	99.86±2.02*	94.68±1.21* **	91.20±1.23* **
Lysis azocasein, E440/(h×mg of tissue)	83.91±0.89	101.49±2.19*	97.54±1.92*	91.26±1.33* **
Lysis azocollagen, E440/(h×mg of tissue)	2.12±0.07	3.26±0.22*	2.89±0.12*	2.71±0.14* **
TFA, E440/(h×mg of tissue)	52.94±1.59	71.60±1.81*	68.71±1.42*	63.14±0.98* **
NFA, E440/(h×mg of tissue)	31.77±1.87	45.26±1.20*	43.89±0.95*	38.20±1.46* **
EFA, E440/(h×mg of tissue)	21.49±0.89	26.34±0.85*	24.83±0.96*	24.94±0.57*

Note. * – reliability of differences compared with the control group of rats;
** – reliability of differences compared with the group of rats with Alzheimer's disease.

After enalapril administration TFA in the cerebral cortex 8.7% decreased, in the hippocampus – 11.8%. At the same time, both EFA and NFA decreased – 7.4 and 12.7% respectively; NFA 15.6% decreased in the hippocampus only. Such effects of unequal value can be explained by phylogenetic peculiarities of the structures and differences of pathobiochemical chains of neuron damage (Anand and Dhikav, 2012).

4. DISCUSSION

Thus, the results of the studies conducted demonstrated increase of the intensity of unlimited proteolysis with Alzheimer's disease of both low molecular and high molecular proteins, which is stipulated by a considerable activation of free radical processes in the structures examined (Labandeira-Garcia et al., 2017). An increased concentration of active oxygen forms is known (Beckhauser et al., 2016) to promote damage of the neuron cellular membranes associated with an increased activity of membrane-bound enzymes including proteolysis enzymes. Proteolytic enzymes splitting peptide bonds of protein molecules are one of the important mechanisms controlling the functions of organs and tissues, and brain in particular (Shakeera & Sujatha, 2019). At the same time,

increased fibrinolysis intensity in rats with neurodegeneration can be considered as a compensatory response to hypercoagulation due to considerable accumulation of pathological peptide, and β-amyloid peptide in particular.

It should be noted that a course administration of carbacetam and enalapril produced certain effect in case of disturbed processes of proteolysis/fibrinolysis of the cerebral cortex and hippocampus of rats with Alzheimer's disease. The results of ours studies obtained are stipulated by the pharmacodynamics properties of the drugs examined. In fact, decreased lysis of low molecular proteins in the hippocampus under carbacetam influence is associated with a modulating effect on GABA-receptors of the given structure. It results in an increased concentration of the intracellular chlorine anion causing hyperpolarization, improving neuron communication and synchronization of neuron populations, and decelerating proteolytic processes (Negah Rahmati et al., 2018). Moreover, smooth muscle tone of the cerebral vessels become normal, hermeticity and selectivity of the hematoencephalic barrier are restored, and non-enzymatic fibrinolytic activity of the cerebral cortex decreases respectively.

Changes of proteolysis/fibrinolysis after enalapril are associated with peculiarities of the mechanism of action of this medicinal agent. Enalapril, as ACE inhibitor, inhibits the complex of NADPH-oxidase, decelerates oxidative stress processes, which is evidenced by our previous studies (Kmet et al., 2019), and microglia inflammatory reaction (Kinney et al., 2018). It results in reduced amount of neurotoxins and pro-inflammatory factors, which are initiators of proteolysis and fibrinolysis. Reduced mediators of inflammation decrease expression of plasminogen activator inhibitors which inhibit tPA, and thus decreasing plasmin activity. In addition to bradykinin decrease, ACE inhibition promotes accumulation of angiotensin 1-7, which is reflected by improvement of the cerebral circulation and normalization of rheological properties of the blood (Gebre et al., 2018; Bennion et al., 2015). It results in deceleration of processes of fibrinogen sedimentation, decrease of inflammation and normalization of vascular permeability. The processes described slow damages and development of vascular dysfunction, and become the protective mechanisms for neurons. Therefore, the results obtained are indicative of reasonability of pathogenic correction by means of modulators of the GABA and renin-angiotensin systems under conditions of Alzheimer's disease development.

5. CONCLUSION

Under conditions of Scopolamine-induced; Alzheimer's disease proteolytic and fibrinolytic activity of the cerebral cortex and hippocampus of rats increases. After carbacetam was administered to rats with Alzheimer's disease during 14 days, collagenolysis and enzymatic fibrinolytic activity in the cerebral cortex decreased, and low molecular proteinolysis – in the hippocampus only. Under enalapril effect proteolysis/fibrinolysis indices decrease in both structures of the brain examined. The results obtained confirm participation of proteolytic and fibrinolytic systems in the mechanisms of neurodegeneration and are indicative of reasonability of pathogenic correction by means of modulators of the GABA and renin-angiotensin systems under conditions of Alzheimer's disease development.

List of abbreviations

ACE - angiotensin converting enzyme
EFA - enzymatic fibrinolytic activity
GABA - gamma-aminobutyric acid
HMP - high molecular proteinolysis
LMP - low molecular proteinolysis
NFA - nonenzymatic fibrinolytic activity
TFA - total fibrinolytic activity
tPA - tissue plasminogen activator

Funding

This study has not received any external funding.

Author's contribution

Conceptualization, O.G. Kmet and N.D. Filipets;
methodology, O.G. Kmet; K.V. Vlasova;
software, O.G. Kmet; T.I. Kmet;
validation, O.G. Kmet and T.I. Kmet;
formal analysis, O.G. Kmet;
investigation, T.I. Kmet;

resources, Y.M. Vepriuk;
datacuration, Y.M. Vepriuk and N.D. Filipets;
writing—original draft preparation, O.G. Kmet;
writing—review and editing, O.G. Kmet and N.D. Filipets;
visualization, O.G. Kmet and Y.M. Vepriuk;
supervision, O.G. Kmet; K.V. Vlasova;
project administration, O.G. Kmet.
All the authors have read and agreed with the final version of the article.

Conflict of Interest

The authors declare no conflict of interest or financial support. All authors contributed to the research and/or preparation of the manuscript.

Informed consent

Written and oral informed consent was obtained from participants included in the study.

Data and materials availability

All data associated with this study are present in the paper.

Peer-review

External peer-review was done through double-blind method.

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