Article

Dipeptide mimetics of nerve growth factor and brain-derived neurotrophic factor, GK-2 and GSB-106 and their cytoprotective properties in the model of oxidative stress

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Abstract: Relevance. At the V.V. Zakusov Research Institute of Pharmacology. Zakusov dimeric dipeptide mimetics of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), GK-2 and GSB-106, respectively, were created. GK-2 and GSS-106 were found to be similar to the corresponding full-length neurotrophins in their mechanism of action and pharmacological properties, including pronounced neuroprotective activity in vitro and in vivo. The aim of this work was to obtain additional data on the cytoprotective properties of GC-2 and GSB-106 using infusoria. Methods. Oxidative stress in Paramecium caudatum was simulated by addition of heavy metal salts (cadmium chloride, lead acetate, copper sulfate, zinc sulfate) to the medium in final concentration of 10 μM. GK-2 or GSB-106 in concentrations from 10-5 to 10-8 M were added to the medium with experimental cells 45 min before the application of oxidative stress initiator. Results. GK-2 and GSB-106 dipeptides in all studied concentrations protected cells from cell death. The maximum neuroprotective effect was shown by dipeptides in concentration of 10-8 M, thus preventing the infusoria death. Conclusion. GK-2 and GSB-106 at a concentration of 10-8 M fully protect Paramecium caudatum from death under oxidative stress induced by heavy metals.

Keywords: nerve growth factor; brain-derived neurotrophic factor; dimeric dipeptide mimetics; Paramecium caudatum infusoria; cytoprotection; oxidative stress induced by heavy metal salts.

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1. Introduction

Neurodegeneration in the brain is a key link in the pathogenesis of a number of widespread diseases, such as cerebrovascular disorders, Alzheimer's and Parkinson's diseases, depression, etc. Disability due to neurodegenerative processes is a serious social and economic problem, so the search for highly effective neuroprotective agents is an urgent task for pharmacology.

Endogenous neuroprotective proteins such as brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) have a high therapeutic potential [1]. However, the clinical use of neurotrophins is limited by their instability in biological fluids, poor ability to penetrate the blood-brain barrier and pleiotropic side-effects (2, 3).

At the V.V. Zakusov Research Institute of Pharmacology. Zakusov based on the beta bends of NGF and BDNF loops 4 dimeric dipeptides, hexamethylenediamide bis(N-monosuccinyl-L-glutamyl-L-lysine (GC-2) and hexamethylenediamide bis(N-monosuccinyl-L-seryl-L-lysine) (GSB-106) were designed and synthesized respectively [Russian patent No 2410392, 2010; US Patent No. 9683014 B2, 2017; Chinese Patent No. 102365294 B, 2016]. GK-2 and GSB-106 have been shown to activate full-length protein-specific tyrosine kinase receptors, TrkA and TrkB, respectively, and possess neuroprotective activity in vitro at micro-nanomolar concentrations in various cellular models, including oxidative stress models [46]. The neuroprotective activity of GC-2 and GSB-106 was confirmed in vivo in a model of extensive ischemic stroke caused by transient middle cerebral artery occlusion in rats [7-9]. For GK-2, it has been shown to be devoid of the major side-effects typical of NGF, namely it does not cause hyperalgesia and weight loss [6].

To obtain additional data on cytoprotective properties of GK-2 and GSB-106 dipeptides, it was of interest to study them in the model of oxidative stress in infusoria [10, 11]. Oxidative stress

is known to be one of the main mechanisms of neuronal damage in various pathologies. Heavy metal salts [11-13], exotoxicants potentially dangerous for all living organisms, are widely used to model oxidative stress both in vitro and in vivo. Ions of lead, cadmium, zinc and other heavy metals can initiate the generation of excessive amounts of reactive oxygen species [13-15], the increased level of which in the cell triggers chain reactions of oxidative degradation of biomolecules.

Unicellular organisms, infusoria in particular, represent a convenient model organism for pharmacological studies, because in this case the advantages inherent to the use of cell culture are supplemented by the fact that in this case the test system is both a single cell and an integral organism. It should be noted that for infusoria, as for other unicellular organisms, there are no data in the literature on the presence of tyrosine kinase receptors similar to those in vertebrates, which could mediate the pharmacological effects of GC-2 and GSB-106 dipeptides. However, neurotrophin-like growth factors regulating survival and proliferation have been found in infusoria [16, 17], suggesting the presence of similar receptor systems.

2. Patients and Methods

The work was performed on a culture of Ramecium caudatum, one of the most commonly used test objects for laboratory studies aimed at determining the direct effect of chemical compounds. Paramecium cell culture was grown on Lozin-Lozinsky medium with the addition of nutrient medium containing Saccharomyces cerevisiae yeast. Cells taken in the log-phase of growth were incubated at 24 ± 2 °C, pH = 6.8-7.0.

The oxidative stress was modeled [10] by adding 1 ml of aqueous solution of one of metal salts (cadmium chloride, lead acetate, copper sulfate, zinc sulfate) to 1 ml of medium containing Paramecium caudatum infusoria at final concentrations of 1; 5; 10 and 15 μM. The duration of incubation of cells in medium containing heavy metal salt was 15 min, 30 min, 45 min, 1 hour, 2 hours, 6 hours. 45 min before the addition of the oxidative stress initiator, 1 ml of GC-2 or GSB-106 solution in concentrations from 10-5 to 10-8 M was added to the medium containing experimental cells. The active concentrations of HA-2 and GSB-106 were chosen based on the previous experiments [4, 5, 18].

The pH of the medium was measured at all stages of the experiment using a Kelilong PH-221 pH-meter controller. Cell number, intensity of cell division, nature and speed of infusoria movement, and changes in cell shape were recorded. The number of cells was determined under a microscope at 7×10 magnification with video recording by counting their total number in 1 ml of culture.

The results are presented as arithmetic mean ± standard error of the mean. After testing the distribution for normality, the significance of differences between the groups was assessed using Student's t-test. The differences were considered significant at $p < 0.05$.

3. Results

Under the influence of heavy metal ions the cell number was markedly decreased, the most pronounced effects were observed at the concentrations of 10-15 μM salts of heavy metals (Fig.1). A number of morphological changes occurred in the cell, including reorganization of cytoskeleton structures leading to cell death. Swelling of cytoplasm organelles was observed, which led to the rupture of the paramecium cell membrane.

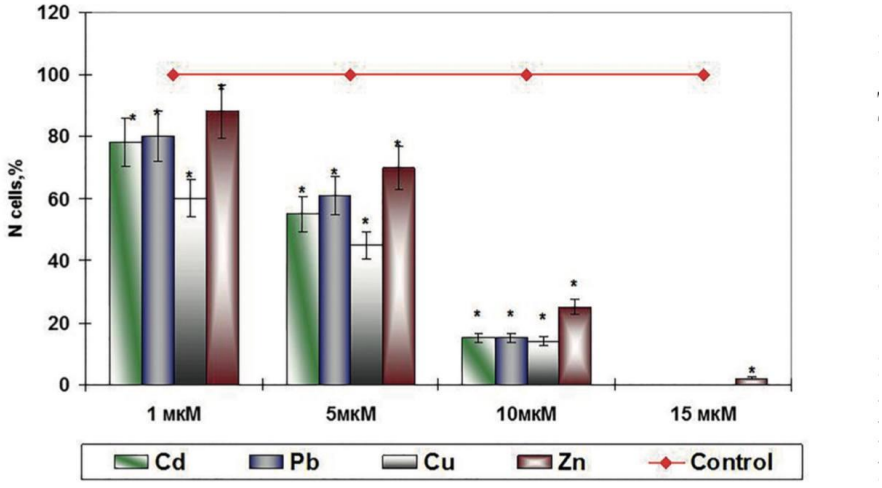

Figure 1. Effect of heavy metal salts on the survival rate of Paramecium caudatum cells after 6 h incubation Notes: Heavy metal salts (cadmium chloride, lead acetate, copper sulphate, zinc sulphate) in final concentrations of 1; 5; 10 and 15 μM were added to the medium and infusoria. The duration of incubation was 6 hours. The abscissa axis shows different concentrations of metal salts; the ordinate axis shows the number of surviving cells in % of the intact control. * - $p < 0.05$ compared to control (Student's t-test).

A decrease in the number of experimental cells as a result of destructive membrane pathology was indicative of the intensification of free-radical oxidation processes caused by heavy metal ions. After 6 h of incubation with heavy metal salts solutions (10 μM), the number of surviving cells in Ramecium caudatum culture was about 15 to 25% of the passive control (without damage) (maximum number in the medium with copper sulfate).

Dipeptides GC-2 and GSB-106 in all concentrations studied protected cells from heavy metal-induced death. The maximum neuroprotective effect of dipeptides was observed in the concentration of 10-8 M (Fig. 2). At this concentration, the compounds studied almost completely prevented cell death of infusoria even after 6 h of incubation with heavy metal salts (10 μM) (see Fig. 2).

The efficacy of GC-2 and GSB-106 in this model suggests that Paramecium caudatum has receptor systems similar to the tyrosine kinase receptors of vertebrates, which is consistent with the literature data on the presence of growth factors in infusoria that regulate survival and proliferation [16, 17].

5. Conclusions

Thus, we found that the dipeptide mimetics NGF and BDNF, respectively GC-2 and GSB-106, at a concentration of 10-8 M fully protect Paramecium caudatum cells from death under oxidative stress caused by heavy metal salts (cadmium chloride, lead acetate, copper sulfate, zinc sulfate).

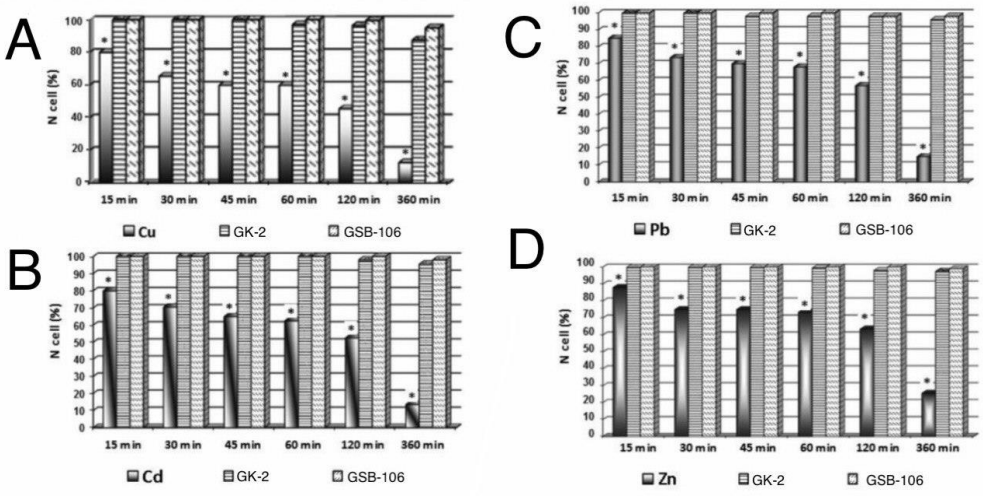

Figure 2. Effect of dimeric dipeptide mimetics NGF and BDNF, respectively GC-2 and GSB-106 at concentrations of 10-8 M on cell survival under oxidative stress induced by heavy metal salts (copper sulfate, cadmium chloride, lead acetate, zinc sulfate) (10 μM) 15, 30, 45, 60, 120 and 360 min after incubation

Notes: A, oxidative stress was induced by copper sulfate; B, oxidative stress was induced by cadmium chloride; oxidative stress was induced by lead acetate; D, oxidative stress was induced by zinc sulfate. GC-2 and GSB-106 were added to the medium 45 min before the toxin. The abscissa axis shows the time of incubation with heavy metal salts; the ordinate axis shows the number of surviving cells in % of the intact control. * - $p < 0.05$ compared to control (Student's t-test).

Application of artificial intelligence:

The article is written without the use of artificial intelligence technologies.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacology & Therapeutics. 2013; 138(2):155–175.

2. Aloe L, Rocco ML, Bianchi P, Manni L. Nerve growth factor: from the early discoveries to the potential clinical use. Journal of Translational Medicine. 2012;10(1):239.
3. Dunkel P, Chai CL, Sperlágh B, Huleatt PB, Mátyus P. Clinical utility of neuroprotective agents in neurodegenerative diseases: current status of drug development for Alzheimer's, Parkinson's and Huntington's diseases, and amyotrophic lateral sclerosis. Expert Opinion on Investigational Drugs. 2012; 21(9):1267–1308.
4. Antipova TA, Gudasheva TA, Seredenin SB. In vitro study of neuroprotective properties of GK-2, a new original nerve growth factor mimetic. Bulletin of Experimental Biology and Medicine. 2011; 150 (5): 607–9. (in Russ).
5. Gudasheva TA, Tarasyuk AV, Pomogaybo SV. Design and synthesis of dipeptide mimetics of brain-derived neurotrophic factor. Bioorganic chemistry.2012; 38 (3): 280–90. (In Russ).
6. Gudasheva TA, Povarnina PY, Antipova TA, Firsova YN, Konstantinopolsky MA, Seredenin SB. Dimeric dipeptide mimetics of the nerve growth factor Loop 4 and Loop 1 activate TRKA with different patterns of intracellular signal transduction. Journal of Biomedical Science. 2015; 22(5):106.
7. Gudasheva TA, Povarnina P, Logvinov IO, Antipova TA, Seredenin SB. Mimetics of brainderived neurotrophic factor loops 1 and 4 are active in a model of ischemic stroke in rats. Drug Design, Development and Therapy. 2016; 10:3545–3553.
8. Povarnina P, Gudasheva TA, Seredenin SB. Dimeric dipeptide mimetics of NGF and BDNF are promising agents for post-stroke therapy. Journal of Biomedical Science. 2018; 11(5):100–107.
9. Seredenin SB, Povarnina PY, Gudasheva TA. An experimental evaluation of the therapeutic window of the neuroprotective activity of a low-molecular nerve growth factor mimetic GK-2. Journal of Neurology and Psychiatry named after S. S. Korsakov. 2018; 118 (7): 49. (in Russ).
10. Karpukhina OV, Gumargalieva KZ, Inozemtsev AN. The effect of antioxidant compounds on oxidative stress in unicellular aquatic organisms. In: On the borders of physics, chemistry, biology, medicine and agriculture. Research and Development. Torun: Institute for Engineering of polymer materials and Dues. 2014: 145–151.
11. Morgunov IG, Karpukhina OV, Kamzolova SV, et al. Investigation of the effect of biologically active threo-Ds-isocitric acid on oxidative stress in Paramecium caudatum. Preparative Biochemistry & Biotechnology. 2018;48(1):1–5.
12. Simmons SO, Fan CY, Yeoman K, Wakefield J, Ramabhadran R. NRF2 Oxidative Stress Induced by Heavy Metals is Cell Type Dependent. Current Chemical Genomics. 2011; 5:1–12.
13. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Current Topics in Medicinal Chemistry. 2001; 1 (6): 529–539.
14. Flora SJS, Mittal M, Mehta A. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian Journal of Medical Research. 2008; 128 (4): 501– 523.
15. Pryor WA. Oxy-radicals and related species: their formation, lifetimes, and reactions. Annual Review of Physiology. 1986; 48: 657–667.
16. Tanabe H, Nishi N, Takagi Y, Wada F, Akamatsu I, Kaji K. Purification and identification of a growth factor produced by Paramecium tetraurelia. Biochemical and Biophysical Research Communications. 1990; 170 (2): 786–792.
17. Rasmussen MI, Wheatley DN. Purification and characterisation of cell survival factor 1 (TCSF1) from Tetrahymena thermophila. Journal of Cell Communication and Signaling. 2007; 1(3-4): 185–193.
18. Gudasheva TA, Antipova TA, Seredenin SB. Novel lowmolecular-weight mimetics of the nerve growth factor. Doklady Biochemistry and Biophysics. 2010; 434: 262-5. (In Russ).