ИММУНОМОДУЛИРУЮЩАЯ И НЕЙРОТРОПНАЯ АКТИВНОСТИ СИНТЕТИЧЕСКИХ ПЕПТИДОВ НА МОДЕЛИ ЧЕРЕПНО-МОЗГОВОЙ ТРАВМЫ У КРЫС

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Резюме. Лечение последствий черепно-мозговой травмы (ЧМТ) остается одной из актуальных проблем современной медицины. Для повышения эффективности лечения посттравматических осложнений рекомендуют различные препараты, обладающих нейропротекторной и нейрорепаративной активностью, в том числе пептид с нейромодуляторной активностью Семакс (Semax).

Цель настоящего исследования – определить наличие нейро- и иммунопротекторных свойств у синтетического пептида PR5, составленного из фрагментов пролин-богатых антимикробных пептидов.

Работа выполнена на крысах-самцах породы Wistar массой 300-350 г. В качестве модели механической травмы головного мозга использовали модель «падающего груза», в собственной модификации, вызывающую в основном диффузное повреждение мозга. Использовали синтезированный пептид PR5, составленный из фрагментов известных пролин-богатых пептидов нейтрофилов животных, и пептидный препарат Semax в виде 1%-ного водного раствора. Препараты вводили интраназально через 1 час после ЧМТ, затем 2 раза в день в течение 4 дней в дозе 100 мкг/кг массы тела. Контрольные животные, которые получали физиологический раствор в том же режиме, что и пептидные препараты.

ЧМТ приводила к значимому снижению массы тела на 14-е сутки после ЧМТ, однако у крыс, получавших пептидный препарат Semax, падение массы тела было существенно меньшим, чем у контрольных животных, а препарат PR5 полностью предотвращал падение массы тела после ЧМТ. На 7-е сутки после ЧМТ угнеталась пролиферативная активность лимфоцитов и снижалась цитотоксичность NK-клеток. У животных, пролеченных пептидными препаратами Semax и PR5, существенного угнетение цитотоксичности NK-клеток не наблюдалось, а пролиферативная активность лимфоцитов восстанавливалась до показателей контрольных животных к 14-м суткам после ЧМТ. Оба использованных пептидных препарата способствовали более высокой локомоторной активности на 7-е сутки., а у животных, пролеченных пептидом PR5, к 14-м суткам этот вид активности достигал параметров контрольных животных. Снижение продолжительности фризинга в группах, пролеченных пептидными препаратами, свидетельствует о наличии седативного эффекта.

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Пептидный препарат PR5 был активен в данной серии экспериментов, показав иммунотропную и нейропротекторную активность, сопоставимую с препаратом Semax. Дальнейшие исследования, направленные на подтверждение выявленных видов активности пептидного препарат PR5, могут обосновать его перспективы для клинического использования как нового ноотропного агента.

Ключевые слова: регуляторные пептиды, черепно-мозговая травма, стресс, активность лимфоцитов, поведенческие тесты, нейротропный эффект

IMMUNOMODULATORY AND NEUROTROPIC ACTIVITIES OF SYNTHETIC PEPTIDES IN A MODEL OF BRAIN INJURY IN RATS

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Abstract. Treatment of consequences of traumatic brain injury (TBI) remains one of the current problems of medicine. To increase the effectiveness of treatment of post-traumatic complications, various drugs are recommended, including the peptide with neuromodulatory activity Semax.

The present study aims to determine the presence of neuro- and immunoprotective properties of the synthetic peptide PR5, composed of fragments of proline-rich antimicrobial peptides.

The work was performed on male Wistar rats weighing 300-350 g. The "falling weight" model of mechanical brain injury was used, which mainly causes diffuse brain damage. The synthesized peptide PR5, composed of fragments of known proline-rich peptides of animal neutrophils, and the peptide preparation Semax in the form of a 1% aqueous solution were used. The drugs were administered intranasally 1 hour after TBI, then twice a day for 4 days at a dose of 100 μg/kg body weight. Control animals received physiological saline in the same regimen as the peptide preparations.

TBI led to a significant decrease in body weight, but in rats receiving the peptide preparation Semax, the decrease in body weight was significantly less than in control animals, and the PR5 preparation completely prevented the decrease in body weight after TBI. After TBI, the proliferative activity of lymphocytes was suppressed and the cytotoxicity of NK cells decreased. In animals treated with peptide preparations, there was no significant suppression of NK cell cytotoxicity, and the proliferative activity of lymphocytes was restored to the level of control animals by day 14 after TBI. Both peptide preparations used contributed to higher locomotor activity, and in animals treated with the PR5 peptide, this type of activity reached the parameters of control animals. The reduction in freezing duration in groups treated with peptide preparations indicates the presence of a sedative effect.

The peptide preparation PR5 was active in this series of experiments, showing immunotropic and neuroprotective activity comparable to the Semax preparation. Further studies aimed at confirming the identified types of activity of the peptide preparation PR5 may justify its prospects for clinical use as a new nootropic agent.

Keywords: regulatory peptides, traumatic brain injury, stress, lymphocyte activity, behavioral tests, neurotropic effect

Introduction

Treatment of consequences of traumatic brain injury (TBI) remains one of the pressing problems of modern medicine. Patients with brain injuries of varying severity often suffer from physical and cognitive impairments for months and years, and there are no standard methods of therapy for these conditions. Various drugs with neuroprotective and neurorepair activity, including regulatory peptides, are used as correctors of post-traumatic complications. Regulatory peptides belong to a group of biologically active substances of peptide nature, having signs of polyfunctionality. Some authors classify regulatory peptides as histohormones, based on their relatively short half-life and participation in regulation processes at the local tissue level [1]. One of the peptides synthesized by the Ashmarin I.P. academician group, Semax, a synthetic analog of the ACTH4-10 fragment, has proven itself as a neuromodulator that stimulates a range of brain functions and is currently approved for the treatment of consequences of strokes, optic nerve atrophy, and some other neurological diseases [2].

Previously, it has been shown that natural antimicrobial peptides such as defensins and protegrins have corticostatic activity and can reduce stress-induced or ACTH-induced increases in corticosterone levels in blood, thereby exhibiting stress-protective effects. The current study focuses on analyzing the effects of a new synthetic peptide in order to identify its neuro- and immunoprotective properties.

The study aims to investigate the regulatory capabilities of the synthetic peptide PR5 compared to Semax on immune cell activity and behavioral parameters before and after traumatic brain injury in experimental rats.

Materials and methods

The study was conducted on male Wistar rats weighing 300-350 g. The animals were housed in vivarium conditions at room temperature with a 12-hour light/dark cycle, free access to water and food, and fed a standard diet in accordance with laboratory animal maintenance standards.

The model of mechanical traumatic brain injury used was the "falling weight" model, also known as the "impact acceleration" model, in our own modification, which mainly causes diffuse brain damage [6]. A weight (460 g) with a blunt surface fell inside a hollow tube (inner diameter of 20 mm) from a height of 60 cm. The distance between the end of the tube and the animal's head was 7 cm. Prior to the injury, the animals received ether anesthesia at a rate of 3-5 mL of medical ether per 1 kg of body weight in a mixture with atmospheric air. The impact zone was located in the central part of the temporal area. The device for applying TBI was assembled at the Department of General Pathology and Pathophysiology of the Institute of Experimental Medicine on the basis of literature data on devices for the "falling weight" model [3].

After the injury, the animals were transferred to a special plastic cage, where they were observed until the restoration of normal behavioral patterns. The experiments were carried out in accordance with the National Standard of the Russian Federation GOST R-53434-2009 "Principles of Good Laboratory Practice" and the Order of the Ministry Health of the Russian Federation dated April 1, 2016 No. 199n "On Approval of the Rules of Good Laboratory Practice". All manipulations performed on animals were reviewed and approved at a meeting of the bioethical commission of the Institute of Experimental Medicine

The PR5 peptide sequence was composed of fragments of known proline-rich peptides of animal neutrophils, including PR-39 (domestic pig neutrophils) and bactenecin ChBac5 (domestic goat neutrophils), with an additional glyproline (Pro-Gly-Pro) at its C-terminus. The peptide was produced via the Fmoc solid phase synthetic approach on a Symphony X peptide synthesizer (Protein Technologies, USA) using a standard synthesis protocol. The purity of the peptide as determined by analytical chromatography was 96-98%. The molecular weight was confirmed by MALDI TOF mass spectrometry. Peptides were dissolved in physiological saline for experiments. Semax, a 1% aqueous solution of peptide, was purchased from a pharmacy.

In the series of experiments investigating the effects of PR5 peptide on TBI, the following experimental groups were formed, each consisting of 6-8 animals: 1 – control animals; 2 – animals subjected to TBI; animals subjected to TBI receiving PR5 peptide (group 3); and animals receiving Semax peptide after TBI (group 4). All peptides were administered intranasally 1 hour after TBI, followed by twice daily administration for 4 days at a dose of 100 μg/kg body weight. Material for analysis was collected on days 7 and 14 after TBI. Animals receiving physiological saline in the same regimen as the peptide groups were used as control.

The cytotoxic activity of spleen lymphocytes against K-562 cells and their ability to proliferate were used as functional parameters of immune cells to assess the effects of TBI. The intensity of lymphocyte proliferation was evaluated using the blast transformation reaction upon addition of Concanavalin A (ConA) and recombinant IL-1β (Betaleukin, produced by the Research Institute Highly Pure Biopreparations, St. Petersburg).

To analyze changes in the functional state of the CNS in animals after TBI, behavioral reactions were recorded using the "Open Field" test and VideoMot 2 software (TSE Systems, Germany). Motor activity was evaluated by changes in total distance traveled and average speed, exploratory activity by the animal's location in different sectors of the open field, vertical motor activity, and anxiety level by the number of grooming and freezing acts. The duration of behavior recording for each animal in the test was 5 minutes.

Statistical analysis of the results was performed using the STATISTICA 7 software package with the Mann–Whitney pairwise comparison test. Differences were considered significant at $p < 0.05$.

Results and discussion

Traumatic brain injury (TBI) was a significant stressor event, as evidenced by the previously shown sharp fluctuations in the level of corticosterone (Cs) in the blood of injured animals [5]. Considering that experienced stress is associated with weight loss [4], the nature of changes in body weight in the studied groups was traced.

The results presented in Figure 1 indicate that TBI led to a significant decrease in body weight, however, in rats receiving the peptide preparation Semax, the decrease in body weight was significantly less than

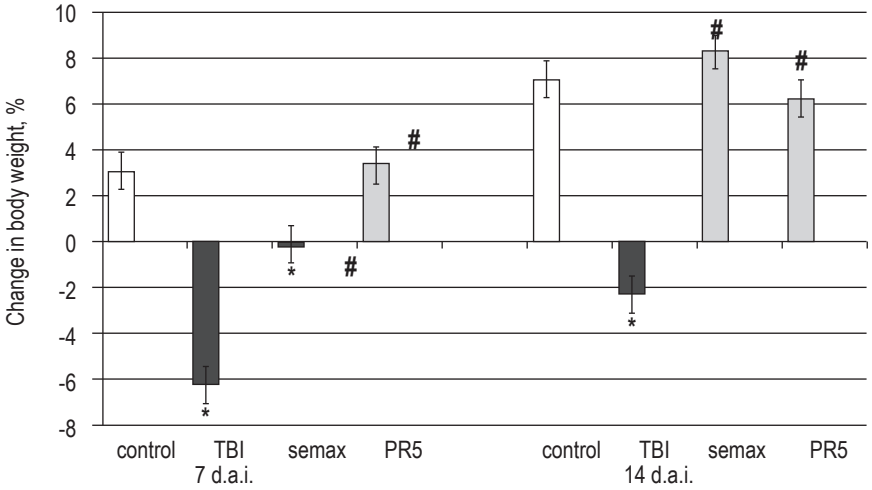

Figure 1. Changes in body weight of animals at 7 days after TBI and administration of the peptide preparations
Note. *, $p < 0.05$ compared to control animals. #, $p < 0.05$ compared to animals after TBI.

in untreated animals at 7 days post-TBI, and the PR5 preparation completely prevented the decrease in body weight in injured animals (Figure 1). In the presented series of experiments, a decrease in body weight of injured animals continued for up to 14 days after TBI. The Semax preparation contributed to the normalization of body weight of injured animals at 14 days post-TBI, while the PR5 peptide prevented stress-induced weight loss at both 7 and 14 days post-TBI.

The impact of traumatic brain injury (TBI) on the functional parameters of immune cells was assessed by means of evaluating the cytotoxic activity of spleen lymphocytes against K-562 cells and their ability to proliferate. The obtained data indicate that after TBI, the cytotoxicity of NK cells decreased at 7 days post-TBI and returned to baseline levels by 14 days post-TBI. In animals treated with the peptide preparations Semax and PR5, there was no significant suppression of NK cell cytotoxicity at 7 days post-TBI (Table 1). Additionally, at 7 days post-injury, there was a significant inhibition of lymphocyte proliferative activity stimulated by suboptimal doses of Con A and enhanced activation of cells by Con A in combination with IL-1β. Administration of Semax and PR5 prevented the inhibition of lymphocyte proliferative

TABLE 1. CYTOTOXIC AND PROLIFERATIVE ACTIVITY OF LYMPHOCYTES AT 7 AND 14 DAYS POST-TBI AND ADMINISTRATION OF PEPTIDE PREPARATIONS

Groups	Cytotoxic activity, %	Proliferative activity, stimulation index	
		Stimulation with Concanavalin A	Stimulation with Concanavalin A and IL-1β
Control	19.8 (18.1-22.8)	1.73 (1.66-2.40)	2.70 (1.90-2.80)
TBI, 7th day	13.3 (8.6-14.3)*	1.28 (0.66-1.56)*	1.50 (0.90-1.66)*
TBI + Semax, 7th day	16.3 (15.7-17.9)#	1.75 (1.45-2.20)	2.20 (1.91-2.50)#
TBI + PR5 7th day	18.8 (16.6-20.0)#	1.66 (1.66-1.93)	2.66 (2.15-2.88)#
TBI, 14th day	18.9 (15.9-19.5)	1.55 (1.35-2.05)	1.67 (1.57-2.30)*
TBI + Semax 14th day	22.0 (19.3-24.0)	1.69 (1.45-2.25)	2.75 (1.96-3.40)#
TBI + PR5 14th day	18.5 (16.1-20.1)	1.66 (1.45-1.88)	1.72 (1.67-2.50)#

Note. *, $p < 0.05$ compared to control animals at the same time of observation. #, $p < 0.05$ compared to injured animals at the same time of observation.

TABLE 2. HORIZONTAL MOTOR ACTIVITY, EXPLORATORY BEHAVIOR RESPONSE (ebr), NUMBER OF GROOMING ACTS, AND DURATION OF FREEZING AT 7 AND 14 DAYS POST-TBI AFTER ADMINISTRATION OF PEPTIDE PREPARATIONS

Groups	Horizontal locomotor activity		EBR	Number of grooming acts	Freezing duration, sec
	distance, m	speed, cm/s			
Control 7th day	17.1 (12.3-22.1)	5.85 (3.55-7.52)	23 (18-27)	8 (4-12)	6 (0-9)
TBI, 7th day	9.6 (7.1-13.5)*	3.16 (2.40-4.40)*	18 (15-23)	9 (6-12)	35 (17-82)*
TBI + Semax, 7th day	10.9 (8.8-13.5)*	3.65 (2.95-4.30)	18 (16-51)	11 (6-16)	16 (7-21)#
TBI + PR5 7th day	14.4 (10.3-18.6)#	4.82 (3.43-6.21)	26 (14-38)#	9 (5-13)	18 (10-24)#
Control 14th day	11.6 (9.6-12.3)	3.78 (3.40-4.11)	25 (23-28)	8 (5-10)	66 (47-84)
TBI, 14th day	8.9 (6.5-10.7)	2.96 (2.15-3.56)	15 (8-123)	3 (1-10)	102 (58-185)*
TBI + Semax 14th day	9.9 (4.7-16.3)	3.32 (1.57-5.45)	19 (8-38)#	8 (3-11)	54 (37-87)#
TBI + PR5 14th day	12.3 (9.6-19.0)#	4.07 (3.22-6.33)	13 (7-30)	4 (1-9)	75 (37-105)

Note. As for Table 1.

activity in injured animals. In untreated animals, inhibition of lymphocyte proliferative activity was observed at 14 days post-TBI when using combined stimulation with Con A and IL-1β, while in animals treated with Semax and PR5, the stimulation index was higher than that in injured animals and did not differ from control animal values. The obtained data indicate the immunoprotective effects of both peptide preparations.

Study of animal behavior in the "Open Field" test showed (Table 2) that horizontal motor activity in terms of length and running speed significantly decreased in injured animals at 7 days post-TBI compared to control animals. However, in animals treated with the PR5 peptide, motor activity was preserved and remained significantly higher than in untreated injured rats. By 14 days post-TBI, horizontal motor activity in animals treated with the PR5 peptide was significantly higher than in injured rats and did not differ from control animal values. The running speed in rats treated with both peptides did not differ significantly from that in control rats.

The exploratory behavior response (EBR) of injured rats was assessed by the number of peeks into "nests" vertical posts (vertical motor activity), and the number of exits into the center of the field.

It was shown that at 7 days post-TBI, the EBR in animals treated with the PR5 peptide was higher than in injured animals, and at 14 days post-TBI, the EBR index was higher in rats treated with the Semax peptide compared to injured animals.

The level of hidden anxiety in rats was judged by the number of grooming acts and the duration of freezing (immobility reaction). There were no changes in the number of grooming acts in the studied groups, however, injured rats significantly differed from control animals in the duration of freezing at 7 days post-TBI. Animals treated with Semax and PR5 showed an increase in the duration of freezing compared to control animals, but the episodes of immobility were significantly less prolonged compared to injured animals. By 14 days post-TBI, the duration of freezing in all peptide-treated animals had returned to control animal parameters, while it increased in injured animals.

Conclusion

Based on the obtained data, it can be concluded that Semax and PR5 preparations prevent post-traumatic weight loss after TBI, restore cytotoxic and proliferative activity of spleen lymphocytes when stimulated with Concanavalin A and IL-1β at 7 days after TBI. Both peptide preparations contribute to higher locomotor activity at 7 days after TBI, and in animals treated with the PR5 peptide, this type of activity reached the parameters of control animals by day 14. The reduction in freezing duration at 7 days after TBI in groups treated with peptide preparations indicates the presence of sedative activity, which is manifested for the Semax preparation at day 14 as well. Thus, the peptide preparation PR5 was active in this series of experiments, showing immunotropic and neuroprotective activity. Further studies aimed at confirming the identified types of activity of the peptide preparation PR5 may justify its prospects for clinical use as a new nootropic agent.

References

1. Ametov A., Shustov S., Khalimov Yu. Endocrinology. A textbook for medical students. Moscow: GEOTAR-Media, 2016. 352 p.
2. Ashmarin I.P., Yeshchenko N.D., Karazeeva E.P. Neurochemistry in tables and diagrams. Moscow: Exam, 2007. 144 p.
3. Bureš J., Burešová O., Huston J.P. Techniques and basic experiments for the study of brain and behavior. Amsterdam; New York: Elsevier Science Publishers B.V., 2nd ed., 1983. 326 p.
4. Muraeva N.A. Influence of chronic stress on body weight and immune organs of experimental animals of early age. *Volgograd Journal of Medical Scientific Research, 2019, no. 4, pp. 3-7.* (In Russ.)
5. Shamova O.V., Lesnikova M.P., Kokryakov VN., Shkhinek E.K., Korneva E.A. Effect of defensins on the blood level of corticosterone and the immune response during stress. *Bull. Exp. Biol. Med., 1993, Vol. 115, pp. 728-731.*
6. Shanin S.N., Fomicheva E.E., Filatenkova T.A., Serebryanaya N.B. Correction of disorders of neuroimmune interactions in experimental traumatic brain injury with recombinant interleukin-2. *Medical Immunology (Russia), 2018. Vol. 20, no. 2, pp. 171-178.* doi: 10.15789/1563-0625-2018-2-171-178.
7. Zhu Q., Hu K., Mulay S. Isolation and structure of corticostatin peptides from rabbit fetal and adult lung. *Proc. Natl Acad. Sci. USA, 1988, Vol. 85, pp. 592-596.*

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