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Fwd: Accepted with Minor Revision (BaliMedJ) The Effect of ACTH4-10Pro8-Gly9-Pro10 on Neurotrophin-3 Expression in Sprague Dawley Rat on Acute Spinal Cord Injury

3 pesan

Gemma Maliawan <gemma.daniswara@gmail.com> 18 September 2023 pukul 12.56
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PENELITI UTAMA : Eko Agus Subagio

UNIT/LEMBAGA/TEMPAT PENELITIAN : Program Doktoral Ilmu Kedokteran Fakultas Kedokteran Universitas Airlangga

DINYATAKAN : LAIK ETIK

Surabaya, 23 April 2014

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Ketua,

Dr. E. Bimo Aksono, M.Kes.,Drh.
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The Effect of *ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}* on Neurotrophin-3 Expression in Sprague Dawley Rat on Acute Spinal Cord Injury

ABSTRACT

Background: Spinal cord injury (SCI) is an injury that causes the most disability, from damage to sensory and motor functions to multiorgan dysfunction. Cells making up the central nervous system (CNS) in humans are known to have a very limited regeneration response that does not allow for complete healing after injury. In addition, neurotrophic factors such as NT-3 play an important role in spinal cord regeneration, which contributes to spinal cord regeneration assisting functional improvement in SCI. ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} which is an analog of the N-terminal fragment (4-10) adrenocorticotropic hormone is one of the neuroprotective compounds that can increase NT-3 levels in brain ischemia. We conducted a study with experimental animals to determine the effect of NT-3 expression on ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} administration in acute compression SCI.

Objective: Determine the effect of giving ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} on expression NT-3 in the spinal cord after mild and severe compression.

Method: We used Sprague Dawley rats with acute compression SCI model using 20 gr and 35 gr aneurysm clips. ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} was administered intranasally to the treatment group and 0.9% NaCl was administered intranasally to the positive control group. Both of the groups then terminated at 3 and 6 hours. Expression of NT-3 was evaluated by immunohistochemical method and statistical calculation conducted in the SPSS Statistics program.

Results: In rats with mild SCI that were given ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}, the NT -3 expression after 3 hours and 6 hours was 14 (12-17) and 10 (7-13). In rats with severe SCI given ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}, the NT-3 expression after 3 hours and 6 hours was 9 (6-11) and 8 (7-10). Intranasal administration of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} showed higher NT-3 expression compared to the group with 0.9% NaCl in mild and severe SCI.

Conclusion: Intranasal administration of *ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}* can increase *NT-3* expression in mild and severe acute SCI at 3 and 6 hours. Expression of NT-3 in the group with ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} mild acute SCI was higher than in the group with severe acute *SCI*. Further research needs to be done to find out the neuroregeneration effect of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} on SCI.

Keywords: spinal cord injury, central nervous system, neurotrophin-3

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INTRODUCTION

Spinal cord injury (SCI) is an injury that causes the most disability, from damage to sensory and motor functions to multiorgan dysfunction. The incidence of SCI in developing countries ranges from 13.1 to 163.4 per one million population with prevalence rates between 490 to 560 per one million population[1]. In Indonesia, 2014 there were 104 SCI cases registered at Fatmawati General Hospital with the most common etiology being traffic accidents and falling from a height[2]

The highest incidence of traumatic SCI occurs in men of productive age with an average age at the time of injury between 32 – 55.4 years, 37 – 47.9 years, and 26.8 – 56.6 years, respectively for North America, Europe, and Asia[3]. The high incidence of SCI at a young age certainly has an impact on the emergence of serious economic loss for families, communities, and countries. Functional defects caused by SCI significantly have an impact on decreasing quality of life and patient life expectancy[4].

Cells making up the central nervous system (CNS) in humans are known to have a very limited regeneration response that does not allow for complete healing after injury[5]. However, with increasing understanding of the pathophysiology of acute traumatic spinal cord injury, it is possible to develop neuroprotective therapies to maximize the functional integrity of the spinal cord

remaining after injury [6]. The most important thing in the treatment of spinal cord injury is how to achieve axonal regeneration in the damaged area[7]. NT-3 is important for the development and maintenance of neuronal populations and promotes differentiation. In addition, NT-3 is also important in the formation of the substantia nigra and pyramidal pathways which are responsible for motor activity and have the best response in corticospinal axon regeneration compared to other neurotrophins, even considered superior[8].

A neuroprotective compound, ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}, also known as heptapeptide Semax an analog of the N-terminal fragment (4-10) of adrenocorticotropic hormone, is known can increase transcription of NT-3 mRNA 24 hours after the occurrence of ischemia in the rat brain cortex[9]. ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} has also shown efficacy in the treatment of vascular disease, allergic and toxic inflammation, and partial atrophy of the human optic nerve[10]. Intranasal administration of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} in rats with SCI showed an anti-inflammatory response that is expected to prevent secondary injury to nerve[11,12]. Our study was aimed to determine the effect of giving ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} on the expression of NT-3 in the spinal cord after mild and severe compression SCI.

MATERIAL AND METHOD

This study used male Sprague Dawley rats, weighing 250-300 g with a spinal cord injury model. Forty-five samples were divided into 9 groups of five samples each. One group became the control group with spinal cord was left uninjured as a baseline, and the rest became the treatment group in which laminectomy at the level of 2nd thoracic vertebra was performed followed by spinal cord compression using an aneurysm clip with a clamping force of 20 g for mild SCI (4 groups) and 35 g for severe SCI (4 groups) in 1 minute. The laminectomy site was then closed with sutures.

Each treatment group was divided into 2 subgroups. Positive control groups were given 0.9% NaCl intranasally and treatment groups were given ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} intranasally at a dose of 300 mg/kg. Then each group was divided again into two groups to be terminated and then myelum transection was performed at 3 hours and 6 hours after compression. The preparations were fixed in formalin and examined for IHC. The NT-3 was calculated per 100 cells using associated anti-monoclonal antibodies and viewed with a light microscope using a 1000x magnification. Cells that showed positive expression gave the results in the form of a brown color image in the cell cytoplasm (Figure 1).

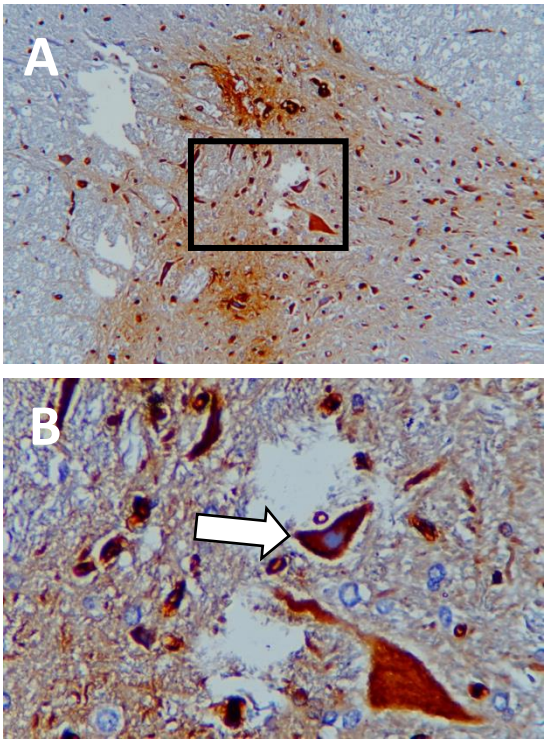

Figure 1. Neurons expressing NT-3 react with anti -NT-3, marked by brown cytoplasm (arrow) by the method IHC. A) 100x magnification. B) 400x magnification in the box marked area in figure A.

STATISTICAL ANALYSIS

Data collection is carried out in a controlled environment with the assumption that all conditions are managed equally and can be controlled. Data of NT-3 level was presented in the form of a relative expression graph. Data normality analysis was performed using Shapiro-Wilk and then the collected data were analyzed using the nonparametric Kruskal-Wallis test with Mann-Whitney difference test.

RESULT AND DISCUSSION

The result of the normality test with the Shapiro-Wilk method showed abnormal data distribution with the Kruskal-Wallis test found significant differences ($p<0,001$) between groups (table 1). In rats with mild SCI that were given ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}, the NT -3 expression after 3 hours and 6 hours was 14 (12-17) and 10 (7-13). In rats with severe SCI given ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}, the NT-3 expression after 3 hours and 6 hours was 9 (6-11) and 8 (7-10). Mann-Whitney test was carried out to pinpoint the differences between groups. Overall NT-3 expressions are shown in figure 2.

SCI causes an imbalance in the microenvironment that can exacerbate and accelerate nerve damage, impairing regeneration and functional recovery[13]. The balance between proneurotrophins and neurotrophins is disrupted after SCI resulting with increase proneurotrophins and decreased neurotrophins which leads to cellular apoptosis, reduces synaptic plasticity, promotes the inflammatory response, and degeneration[14].

Expression of NT-3, BDNF, and NGF is decreasing significantly as early as 6 hours after spinal cord contusion in rat model[15]. Our study showed that NT-3 expression in rats with mild SCI was higher significantly in ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} groups compared to Nacl 0,9% group at 3 hours and 6 hours after compression ($p<0,05$) as shown in table 2. Higher expression of NT-3 was also

achieved significantly in ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} groups with severe SCI compared to NaCl 0,9% group at 3 hours and 6 hours after compression ($p<0,05$) as shown in table 3. ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} is known to act by binding to the melanocortin receptor, such as MC4R which was found in the spinal cord[16]. In vitro studies on astrocytes showed that activation of the MC4R receptor can increase the expression of BDNF through the cAMP-PKA-CREB[17]. A previous study in rats showed that administration of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} intranasally can increase BDNF expression in 3 hours after SCI[18]. The mechanism for increasing NT-3 expression in this study has not been elucidated, but it is possible to have the same mechanism as the increase in BDNF.

In our study, we found significantly higher NT-3 expression in the ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} group with mild SCI than in the ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} group with severe SCI at 3 hours after injury ($p<0,05$) as shown in table 4. Nerve damage that occurs after SCI can result from primary or secondary injury. More severe injury would result in more severe primary damage, causing a more severe imbalance at the tissue, cellular, and molecular levels which of course could affect the expression of NT-3. Intranasal administration of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} in rats with SCI model also showed anti-inflammatory effects by reducing the expression of IL-1, TNF-α, NF-KB, neutrophil, and induced reduction in the apoptotic mechanism through increased ratio of Bcl-2/HSP70[11,12,19].

The peak of NT-3 expression in our study was shown in ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} group 3 hours after mild and severe SCI. Intranasal administration of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} is known to reach the rat brain within 2 minutes post-administration and has a concentration 10-15 times higher than intravenous administration[20]. Another study in rats said that the nootropic and analgesic effects after intranasal administration of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} could be observed 15-30 minutes after administration[21]. Human studies have shown that ACTH4-10 concentrations in

spinal CSF increase within 10 minutes and peak within 30 minutes after intranasal administration, suggesting the possibility of using an extraneuronal pathway for the peptide to reach its target, pass through the intercellular gap in the olfactory epithelium and then diffuse into the subarachnoid space[22].

In this study, no locomotor assessment was carried out in rats, so it is not known the difference in the functional recovery effect of mild and severe SCI after ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} treatment. However, one study showed improved BBB score in rats with the SCI model after intranasal administration of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} [2]. The receptor NT-3 also changes after the occurrence of SCI, so research on the TrkA, TrkB, TrkC, p75NTR, proneurotrophin, PC1, PC2, and furin receptors is also necessary to understand more clearly the action of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} [15].

CONCLUSIONS

Intranasal administration of *ACTH4-10Pro^{8}-Gly^{9}-Pro^{10}* can increase *NT-3* expression in mild and severe acute SCI at 3 and 6 hours. Expression of NT-3 in the group with ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} mild acute SCI was higher than in the group with severe acute *SCI*. Further research needs to be done to find out the neuroregeneration effect of ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} on SCI.

CONFLICT OF INTEREST

No competing interests were declared.

ETHICS CONSIDERATION

Ethics approval has been obtained from Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Airlangga University, Surabaya, with number 325-KE.

FUNDING

This research received no specific grant from any funding agency

AUTHOR CONTRIBUTIONS

All authors contribute to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

Table 1. NT-3 expression test result

Groups	NT3 Expression (cells/visual fields)			P
	Median	Min	Max	
Control	4	2	6	
ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} 3 hours mild	14	12	17	
ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} 6 hours mild	10	7	13	
NaCl 0,9% 3 hours mild	2	1	5	
NaCl 0,9% 6 hours mild	7	5	8	<0,001
ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} 3 hours severe	9	6	11	
ACTH4-10Pro^{8}-Gly^{9}-Pro^{10} 6 hours severe	8	7	10	
NaCl 0,9% 3 hours severe	4	2	6	
NaCl 0,9% 6 hours severe	3	2	5	

Table 2. Comparison of NT-3 expression in mild SCI

Groups	Median	Groups	Median	p
Control	4	Nacl 0,9% 3 hours	2	0,242
		ACTH4-10 3 hours	14	0,009
		Nacl 0,9% 6 hours	7	0,044
		ACTH4-10 6 hours	10	0,009
NaCl 0,9% 3 hours	2	ACTH4-10 3 hours	14	0,009
		Nacl 0,9% 6 hours	7	0.014

		ACTH4-10 6 hours	10	0,009
ACTH4-10 3 hours	14	Nacl 0,9% 6 hours	7	0,008
		ACTH4-10 6 hours	10	0,045
NaCl 0,9% 6 hours	7	ACTH4-10 6 hours	10	0.033

Table 3. Comparison of NT-3 expression in severe SCI

Groups	Median	Groups	Median	p
Control	4	Nacl 0,9% 3 hours	4	0,831
		ACTH4-10 3 hours	9	0,011
		Nacl 0,9% 6 hours	3	0,393
		ACTH4-10 6 hours	8	0,009
NaCl 0,9% 3 hours	4	ACTH4-10 3 hours	9	0,011
		Nacl 0,9% 6 hours	3	0,519
		ACTH4-10 6 hours	8	0.009
ACTH4-10 3 hours	9	Nacl 0,9% 6 hours	3	0,008
		ACTH4-10 6 hours	8	0,517
NaCl 0,9% 6 hours	3	ACTH4-10 6 hours	8	0,009

Table 4. Comparison of NT-3 expression in mild and severe SCI

Kelompok	**Median**	**Kelompok**	**Median**	**p**
ACTH4-10 3 hours mild	14	ACTH4-10 6 hours mild	10	0,045*
		ACTH4-10 3 hours severe	9	0,008*
		ACTH4-10 6 hours severe	8	0,009*
ACTH4-10 6 hours mild	10	ACTH4-10 3 hours severe	9	0,459
		ACTH4-10 6 hours severe	8	0,242
ACTH4-10 3 hours severe	9	ACTH4-10 6 hours severe	8	0,517

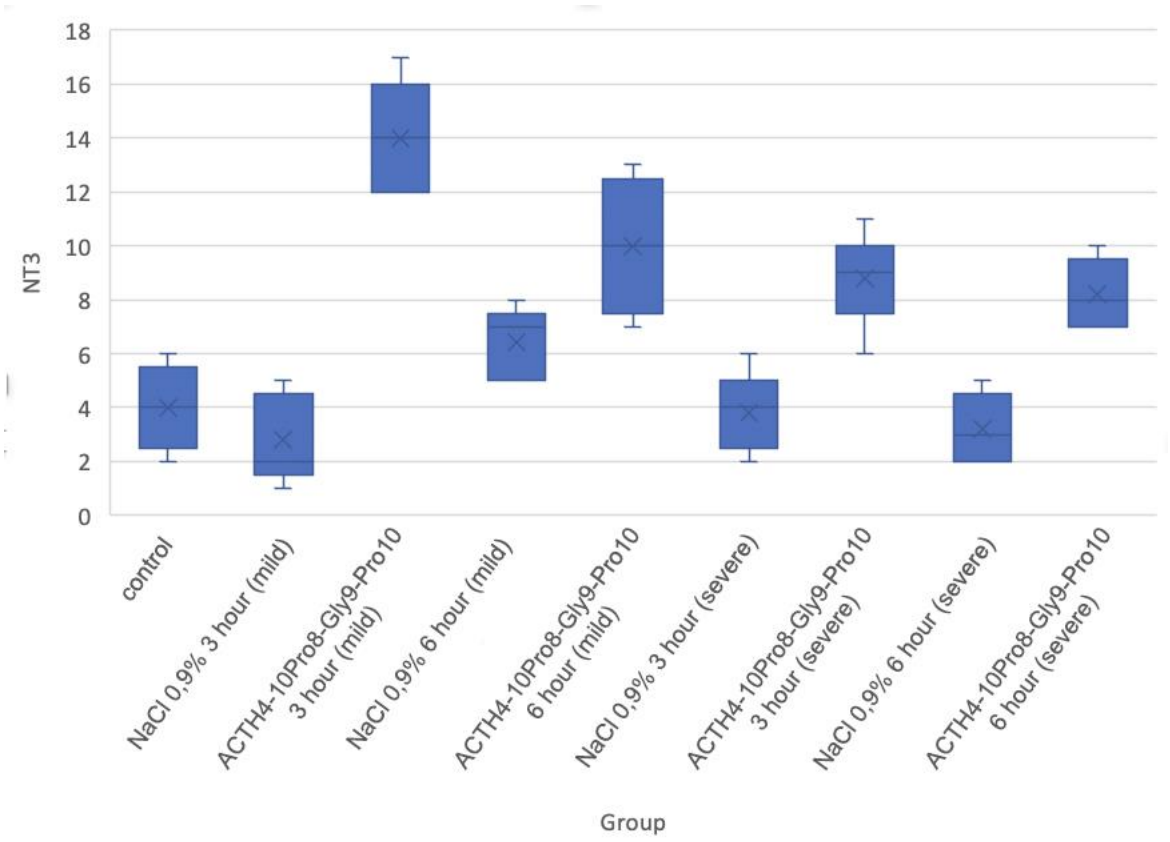

Figure 2. Median value of NT-3 expression

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PENELITIAN BERJUDUL	: Up Regulation BCL-2 Pada Tikus Sprague Dawley yang Mengalami Peningkatan HSP70 Karena *Spinal Cord Injury* Kompresi Akan Menurunkan Derajat Apoptosis
PENELITI UTAMA	: Eko Agus Subagio
UNIT/LEMBAGA/TEMPAT PENELITIAN	: Program Doktoral Ilmu Kedokteran Fakultas Kedokteran Universitas Airlangga
DINYATAKAN	: LAIK ETIK

Surabaya, 23 April 2014

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Letter of Acceptance
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Dear: Made Gemma Daniswara Maliawan[1], Eko Agus Subagio[1*], Budi Utomo[2], Muhammad Arifin Parenrengi[1], Asra Al Fauzi[1], I Ketut Sudiana[3]
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I am very excited to accept your paper entitled:
"The effect of ACTH4-10Pro8-Gly9-Pro10 on neurotrophin-3 expression in sprague dawley rat with acute spinal cord injury."
Your paper will be published in the issue of of Vol. 11 Number 1, 2022.
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Please do not hesitate to contact us if you need anything. It has been a pleasure for us to proofread and edit your work, and we are looking forward to your colleagues and your other papers in the near future.

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