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Original Article

The Effect of ACTH(4-10) PRO8-GLY9-PRO10 Administration towards the Expression of IL-6 and IL-8 in Sprague Dawley Mice with Spinal Cord Injury

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Abstract

Background Spinal cord injury (SCI) is a significant cause of morbidity since it results in inflammation proccess which will lead to necrosis or apoptosis. Inflammatory response to the tissue damage will increase the IL-6 and IL-8. ACTH4–10Pro8-Gly9-Pro10 is a peptide community that has been shown to have a beneficial effect on minimizing the morbidity and increasing the recovery time.

Methods This study is a true experimental laboratory research with a totally randomized method. The subjects were animal models was done with light and extreme compression of spinal cord, respectively.

Results The administration of *ACTH 4–10* in mild SCI for *IL-6* expression in 3 hours observation group didn't pose a significant difference compared with 6 hours observation. The administration of *ACTH 4–10* in severe SCI poses a significantly lower expression level of *IL-6* in 3 hours observation compared with 6 hours. *ACTH 4–10* administration in severe SCI leads to a significantly lower *IL-8* expression in 3 hours observation compared with 6 hours one. In the others hand, there's no significant difference of *IL-8* expression in group receiving *ACTH 4–10* in 3 hours observation compared with the 6 hours one.

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Conclusion The administration of ACTH4–10Pro8-Gly9-Pro10 can reduce the expression of IL-16 and IL-8 at 3 hours and 6 hours after mild and severe SCI in animal models. Future researches are recommended.

Keywords

ACTH4–10Pro8-Gly9-Pro10

spinal cord injury

IL-6

IL-8

Introduction

Spinal cord injury (SCI) carries physical and mental burden to them as it causes permanent motoric and sensory dysfunctions and significantly lower the quality of life. It causes neurological deficits through primary injury, due to irreversible direct and mechanical damage to the myelin during the impact, and secondary injury mechanisms.[1] From the previous study, it is known that 24 hours after trauma is a golden period to conduct a decompression surgery, yet there hasn't been any strict guideline for the benefit of the surgery conducted in acute or later phase of SCI.[2,3] There's a controversial therapy for SCI, which includes the administration of high-dose methylprednisolone that could give more side effects instead of the therapeutical effects.[4] In the future, the treatment approach for SCI will involve blocking the inflammation and progressive pathogenesis of SCI and stimulating the regeneration of neurons.

Inflammation in SCI could induce the apoptosis cascade that leads to cell death. The role of inflammation in SCI's pathophysiology suggested us to test *ACTH(4–10) Pro8-Gly9-Pro10*, a neuromodulator agent that could trigger an anti-apoptosis effect. We conducted an experiment to SCI-induced *Sprague dawley* mice, administered them with intranasal *ACTH(4–10) Pro8-Gly9-Pro10*, and later we collected the myelin to identify *IL-6* and *IL-8* through immunohistochemistry straining.[7] With this study, we aimed to discover more ways to treat inflammation from SCI with the possibility for its future clinical application in increasing the recovery rate and decreasing the morbidity rate.

Material and Methods

This study is a true experimental laboratory study with a completely randomized design. It was conducted in the Animal laboratorium Universitas Airlangga and Pathology Anatomy and Biochemistry Department in the Faculty of Medicine Universitas Brawijaya over the course of 3 months period.

The subjects were divided into control group which did not receive any compression to the spinal cord, while sham and treatment group received spinal cord compression on the T2 level. The control and treatment group were divided into 20 g and 35 g compression groups for mild and severe compression simulation, respectively. The sham group received a placebo using normal saline while the treatment group received *ACTH 4–10* 300 μg/kg (P) through intranasal drops. Spinal cord transection was done for sham and treatment group after 3 hours and 6 hours on the level of injury. The tissues were observed by deparaffinization and immunohistochemistry staining for IL-6 and IL-8, and the levels of *IL-6* and *IL-8* were measured under a light microscope with 400x magnification for all area.

The data were collected in a controlled environment with the same treatment. The levels of *IL-6* dan *IL-8* were presented in a relative expression graph. The normality of the data was tested with shapiro wilks. Normally distributed data will be analyzed using ANOVA followed with post-hoc analysis using Tukey method.

Results

IL-6 Expression in Mild SCI

Table 1. Post Hoc test results for *IL-6* expression in mild SCI with Tukey HSD method ($p < 0,05$)

Mild SCI with NaCl 0,9% in 3 hours and 6 hours observation groups and mild SCI with *ACTH 4–10* in 3 hours observation group showed a significantly higher mean of *IL-6* expression compared with control. However, the administration of *ACTH 4–10* in mild SCI in 3 hours observation group didn't pose a significant difference compared with 6 hours observation.

IL-6 Expression in Severe SCI

Table 2. Post Hoc test results for *IL-6* expression in severe SCI with Tukey HSD method ($p < 0,05$)

IL-6 expression in severe SCI with *ACTH 4–10* in 3 and 6 hours observation groups have a significantly lower mean compared with NaCl 0,9% in 3 and 6 hours observation groups mean. The administration of *ACTH 4–10* in severe SCI poses a significantly lower expression level of *IL-6* in 3 hours observation compared with 6 hours.

IL-8 Expression in Mild SCI

Tabel 3. Post Hoc test results for *IL-8* expression in mild SCI with Tukey HSD method ($p < 0,05$)

There's significant different ($p < 0,05$) in all comparison excluding the *IL-8* expression in severe SCI with NaCl 0,9% in 3 hours observation compared with *ACTH 4–10* 6 hours observation group. *IL-8* expression in severe SCI with *ACTH 4–10* 3 and 6 hours observation groups have a significantly lower mean compared with NaCl 0,9% 3 and 6 hours observation groups. *ACTH 4–10* administration in severe SCI leads to a significantly lower *IL-8* expression in 3 hours observation compared with 6 hours one.

IL-8 Expression in Severe SCI

Table 4. Post Hoc test results for IL-8 expression in severe SCI with Tukey HSD method ($p < 0,05$)

Severe SCI administered with *ACTH 4–10* has a significantly lower *IL-8* expression level compared with the ones given with NaCl 0,9% either in 3 or 6 hours observation groups. There's no significant difference of *IL-8* expression in group receiving *ACTH 4–10* in 3 hours observation compared with the 6 hours one.

Discussion

Spinal injury could lead to assorted injuries, including spinal cord injury (SCI). The clinical manifestation of SCI could vary from spinal cord commotion, contusion, laceration, hemorrhage, compression, to transection. SCI is divided into two process involving a

mechanical primary injury. The process of injury leads to an inflammation and apoptosis.[8] Programed cell death leads to demyelination and axonal degeneration of the injury. Primarily, cell death results from primary injury then continues to secondary injury. Those two mechanisms are mediated by several inflammation, free radicals inducing cell deaths, and glutamate excitotoxicity.[5] Cytokines pathway modulates the central nervous system's inflammation by stimulating the other inflammatory cytokines, chemokines, nitric oxide, and reactive oxygen species.[9] Cytokines in leukocytes pathway that cause cell death are also the ones we studied here, which are *IL-6* and *IL-8*.

Vascular damage by SCI harms the blood-brain barrier (BBB) to which the injury's location is quickly infiltrated by neutrophils. This process majorly contributes to the progression of secondary injury, which the primary injury happens in the first 3–24 hours after SCI. *IL-6* and *TNF-a* are the first pro-inflammatory cytokines released in the first 30–45 of the injury. Around the injured tissue, the production of *IL-6* and *TNF-a* is abundantly significant.[10] *TNF-a* modulates the apoptosis of spinal cord neurons in mice through glutamate pathway and it was reported that the administration of *TNF* antagonists decreases the development of inflammation and tissue damage caused by SCI.[11]

The increase of *IL-1* cytokine family, like *IL-1a* was reported as a clear evidence that they have an important role in inducing inflammation caused by SCI.[8,12,13] Central nervous system gives a response to the inflammation in SCI initiated by immune cells from peripheral tissue and activates glial cells that proliferate and migrate to the injury site after SCI.[10] T cells have an essential role in activating macrophages and modulating cellular immune response. Macrophages and microglial cells contribute in the pathogenesis of secondary injury and cytokine release in inflammation, *TNF-α, IL-1*, *IL-6*, *IL-8* dan *IL-10*.[8] Thus, *TNF-a* and *IL-1* have an important role in apoptosis caused by SCI and it's mediated by a cytokine pathway.

Surgical treatment in SCI is aimed to increase the quality of life, whereas the prevention of secondary treatment is aimed to at least avoid further damage that could lead to a permanent and long-term disability that deteriorate patient's quality of life. The administration of *ACTH(4–10) Pro8-Gly9-Pro10* by blocking the *M2 receptor* is expected to decrease the apoptosis in SCI so it could be considered as a therapy regiment in addition to indicated surgical treatment. The administration of *ACTH(4–10) Pro8-Gly9-Pro10* is given by intranasal drops. It works by a diffusion through olfactory tract's perineural cavity and trigeminal nerve branch, retrograde axonal transport, nose capillary microcirculation, until it reaches the central nervous system. In 1–4 minutes, it arrives to the blood-brain barrier and cerebrospinal fluid (CSF), and the effect lasts to 20–24 ours even after the compound has already been eliminated.[14] It is known that secondary lesion happens in 24 hours after the primary lesion so the treatment is given as early as less than 24 hours after the primary lesion.

IL-6 Expression in Acute Spinal Cord Compression Injury

Based on the results data, in a mild SCI group administered with *ACTH(4–10) Pro8-Gly9-Pro10*, there's a statistically significant difference in 3 hours administration compared with 6 hours in the expression of *IL-6*, both mild and severe SCI. This shows that the administration time of *ACTH(4–10) Pro8-Gly9-Pro10* has a great impact in the expression of inflammatory cytokines. It can be concluded that early administration of *ACTH(4–10) Pro8-Gly9-Pro10* in both mild and severe SCI is expected to modulate inflammation leading to the decrease of secondary lesion of neurons and glial cells from the injury. It is also concluded that in SCI, the early administration, less than 3 hours, is expected to help modulate pro-inflammatory cytokines because of the significant gap of *IL-6* expression compared with the control group.

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This finding is suitable with the existing theories and previous studies, *ACTH(4–10) Pro8-Gly9-Pro10* administration as an anti-inflammation drug that prevents secondary lesion by repairing neurons, blocking the *M2 receptor* activity, dan decrease the level of anti-inflammatory cytokines.[8] There's a previous study showing a drastic increase of *IL-6* expression and its receptor during an acute phase of SCI. This is linked with the function of *IL-6* that could induce the differentiation of neural stem cell into astrocyte that leads to scar formation (glial scar).[15] Another experiment conducted the suppression of *IL-6* where in vitro study shows the suppression of astrocytic differentiation whereas in vivo study shows the inhibition of astrogliosis. The depletion of *IL-6* in SCI-induced animals also shows the decrease of inflammatory cells in site injury and the decrease of scar formation severity and gives a better neurological repair in SCI-induced mice.[15]

IL-8 Expression in Acute Spinal Cord Compression Injury

Trauma in spinal cord plays a role in increasing the level of *IL-8* that serves a function as a chemo attractant that induces the migration of inflammatory cells like neutrophils to the inflammation site.[16] *IL-8* is produced by macrophage and somatic cell who regulate neutrophil and T cell. *IL-8* has a peak level in the first 24 hours in SCI. This is caused by the activation of microglial and neutrophils infiltrating the parenchyma cells.[8]

Based on the results of this study, in mild SCI group administered with *ACTH(4–10) Pro8-Gly9-Pro10,* there's a statistically significant difference in 3 hours administration compared with 6 hours in the expression of *IL-8* both in mild and severe SCI, showing that the time administration of *ACTH(4–10) Pro8-Gly9-Pro10* also has a major role in the inflammatory cytokines expression.

This finding is linked to the discovery saying that glucocorticoids have a role in blocking *IL-8*. The level of cortisol is inversely proportional with *IL-8* and other cytokines. It is concluded that ACTH could indirectly decrease the level of *IL-8* through cortisol pathway.[17]

Conclusion

ACTH 4–10 could decrease the expression of one or more of *IL-6* and *IL-8* in SCI-model animals through spinal cord compression, both mild and severe, and both at 3 hours and 6 hours time. This effect is majorly found in a severe compression of the spinal cord as its level increased on a higher level compared with the mild compression. *ACTH 4–10* might be a viable option to be studied further specifically for SCI in reducing glial formation and irreversible inflammatory damage. Further experiments are needed for analyzing the mechanism of action of *ACTH 4–10* in inhibiting inflammation in SCI.

Disclosure

The authors declare no conflict of interest regarding this study.

Statement of Ethical Principles for Medical Research Involving Human Subjects

This study has not involving human subject

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Table 1 Post Hoc test results for IL-6 expression in mild SCI with Tukey HSD method ($p < 0,05$)

Group	Mean	Standard Deviation	Group	Mean	Standard Deviation	p
Control	3,00	1,63	3 hours SCI	10,43	3,05	0,001*
			6 hours SCI	13,43	3,60	0,001*
			3 hours SCI + *ACTH 4–10*	8,43	2,51	0,005*
			6 hours SCI + *ACTH 4–10*	10,71	1,89	0,001*
3 hours SCI	10,43	3,05	6 hours SCI	13,43	3,60	0,235
			3 hours SCI + *ACTH 4–10*	8,43	2,51	0,621
			6 hours SCI + *ACTH 4–10*	10,71	1,89	1,000
6 hours SCI	13,43	3,60	3 hours SCI + *ACTH 4–10*	8,43	2,51	0,011*
			6 hours SCI + *ACTH 4–10*	10,71	1,89	0,326
3 hours SCI + *ACTH 4–10*	8,43	2,51	6 hours SCI + *ACTH 4–10*	10,71	1,89	0,496

Table 2 Post Hoc test results for IL-6 expression in severe SCI with Tukey HSD method ($p < 0,05$)

Group	Mean	Standard	Group	Mean	Standard	p

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		Deviation			Deviation	
Control	2,86	1,07	3 hours SCI	18,71	2,75	0,001*
			6 hours SCI	27,71	1,70	0,001*
			3 hours SCI + *ACTH 4–10*	10,71	1,38	0,001*
			6 hours SCI + *ACTH 4–10*	16,00	2,31	0,001*
3 hours SCI	18,71	2,75	6 hours SCI	27,71	1,70	0,001*
			3 hours SCI + *ACTH 4–10*	10,71	1,38	0,001*
			6 hours SCI + *ACTH 4–10*	16,00	2,31	0,093
6 hours SCI	27,71	1,70	3 hours SCI + *ACTH 4–10*	10,71	1,38	0,001*
			6 hours SCI + *ACTH 4–10*	16,00	2,31	0,001*
3 hours SCI + *ACTH 4–10*	10,71	1,38	6 hours SCI + *ACTH 4–10*	16,00	2,31	0,001*

Tabel 3 Post Hoc test results for *IL-8* expression in mild SCI with Tukey HSD method ($p < 0,05$)

Group	Mean	Standard Deviation	Group	Mean	Standard Deviation	p
Control	3,43	0,98	3 hours SCI	12,86	1,86	0,001*
			6 hours SCI	18,57	1,90	0,001*
			3 hours SCI + *ACTH 4–10*	7,57	2,07	0,001*
			6 hours SCI + *ACTH 4–10*	12,14	1,68	0,001*
3 hours SCI	12,86	1,86	6 hours SCI	18,57	1,90	0,001*
			3 hours SCI + *ACTH 4–10*	7,57	2,07	0,001*
			6 hours SCI + *ACTH 4–10*	12,14	1,68	0,938
6 hours SCI	18,57	1,90	3 hours SCI + *ACTH 4–10*	7,57	2,07	0,001*

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			6 hours SCI + *ACTH 4–10*	12,14	1,68	0,001*
3 hours SCI + *ACTH 4–10*	7,57	2,07	6 hours SCI + *ACTH 4–10*	12,14	1,68	0,001*

Table 4 Post Hoc test results for *IL-8* expression in severe SCI with Tukey HSD method ($p < 0,05$)

Group	**Mean**	**Standard Deviation**	**Group**	**Mean**	**Standard Deviation**	**p**
Control	3,43	1,99	3 hours SCI	28,71	6,42	0,001*
			6 hours SCI	30,14	4,14	0,001*
			3 hours SCI + *ACTH 4–10*	11,00	2,65	0,009*
			6 hours SCI + *ACTH 4–10*	14,57	2,64	0,001*
3 hours SCI	28,71	6,42	6 hours SCI	30,14	4,14	0,958
			3 hours SCI + *ACTH 4–10*	11,00	2,65	0,001*
			6 hours SCI + *ACTH 4–10*	14,57	2,64	0,001*
6 hours SCI	30,14	4,14	3 hours SCI + *ACTH 4–10*	11,00	2,65	0,001*
			6 hours SCI + *ACTH 4–10*	14,57	2,64	0,001*
3 hours SCI + *ACTH 4–10*	11,00	2,65	6 hours SCI + *ACTH 4–10*	14,57	2,64	0,443

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