



The Effect of ACTH 4-10 ProGlyPro as Anti-inflammatory on Astrocyte Cell Repair in Spinal-Cord-Injured Mouse by Assessing Locomotor Function

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Abstract

Spinal Cord Injury could lead to immobility, paralyze, financial burden due to the high cost of treatments, even emotional disturbance, and distress. It has been a health problem over the years. The primary goal in treating Spinal Cord Injury (SCI) is to prevent secondary lesions of inflammation, thus prevent further disability. A synthetic analog of ACTH (ACTH 4-10 ProGlyPro) does not have hormonal activity, but it stimulates expression of BDNF (Brain-derived Neurotropic Factor), which showed to be a potent modulator of synaptic plasticity in the astrocytes. This research wanted to focus on ACTH 4-10 ProGlyPro's effect which acts as an anti-inflammation, and improvement of motoric function upon treatment with those drugs in an animal study.

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This is an experimental study, which performed by damaging the spinal cord of the white female Wistar mouse at the level of T9 to T11, with a 26 grams-clamp. Then the mouse was separated into four treatment groups, which three were given ACTH 4-10 ProGlyPro in 1 hour, 3 hours, and 6 hours Post compression respectively; and one control group without given ACTH 4-10 ProGlyPro. The motoric function of each mouse was observed using Basso, Beattie, Bresnahan scale at the 1st, 7th, 14th, 21st, and 28th-day post compression. ANOVA analysis shows that the differences of BBB scale between groups were significant, except for day one. We could also see the detail in LSD calculation, where significance was at 0.137 between control and ACTH 4-10 ProGlyPro-1 hour after the given post compression group, 0.08 between control and ACTH 4-10 ProGlyPro 3 hours post Compression, and 0.347 between the control and 6 hours ACTH 4-10 ProGlyPro group. On 7th, 14th, 21th, and 28th day, the BBB scale was statistically significant between control and treatment groups, with the highest mean difference 5.875, 7.625, 8.5, and 8.5 on each consecutive observation day. But the difference within the treatment of ACTH 4-10 ProGlyPro groups was not significant, as shown in the comparison tables and the Mean plots. This study showed a significant effect of ACTH 4-10 ProGlyPro in mouse's motoric function after compression when compared to the control group. But the difference in the timing of ACTH 4-10 ProGlyPro first dose has no statistical significance.

Keywords: ACTH 4-10 ProGlyPro; Synthetic analog of ACTH; Spinal Cord Injury; Locomotor function.

1. Introduction

The nervous system is the backbone of every living individual. Many believed that once neurons are dead, they could not be revived. Spinal cord plays a significant role in our central nervous system; it connects the brain to the rest of the body. Injury to the spinal cord could jeopardize this relationship and leads to immobility, paralyze, financial burden due to the high cost of treatments, even emotional disturbance and distress [1, 2].

Spinal Cord Injury has been a health problem over the years. Since the seventies, there were 11.000 new cases every year in the US. Incidence rate were 40 in 1 million of the population. From 1973 to 1979, people sustained from Spinal Cord Injury (SCI) aged between 16 to 30 years, with an average of 28.7 years old. As life expectancy has improved from time to time, the average age of people with SCI became 40.2 years old in 2005. The first and most frequent cause of SCI was a traffic accident, especially the ones involved in a motorcycle (around 42.1%). Though Indonesia's record hasn't as advanced, it has been expected to be as high. By Indonesian Statistical Bureau there is a significant high inflation of motorcycle riders in Indonesia over the years, they reached the number of 84.73 million in the year of 2013 [3]. Other causes of SCI, which could raise from falls incidents, domestic and social violence (such as a gunshot), and physical exercise injury or sports injury. Before 1980, gun-shot wound responsible for 13.3% cause of SCI. In 1990-1999, it increased to 24.8%, but lowered to 15.1% in 2005 [4].

The improvement in medical and surgical rehabilitation has dramatically enhanced the quantity and quality life of the patients who suffer from acute or chronic SCI [5,6]. But parenchymal damage due to secondary lesions in spinal cord inflammation gives significant consequences to neural damage and long-term disability [7, 8, 9].

From previous literature, a lot of studies has been conducted on stem cells, corticosteroid, melanocortin hormone, and cerebrolysin; in a purpose to stop the formation of secondary lesions [10, 11]. ACTH has a very similar structure to melanocortin, but its' function on SCI has not been evaluated [12]. Unfortunately, ACTH has one fragment that has a petite stability inside the organism, Acton (Met-Glu-His-Phe-Arg-Trp-Gly) [13, 14]. Therefore, there was a synthetic analog of ACTH that substitute action into Met-Glu-His-Phe-Pro-Gly-Pro mutation. This variation duration of action can reach up to 20-24 hours, which 50 times longer than ACTH or melanocortin [15]. Furthermore, this synthetic analog of ACTH (ACTH 4-10 ProGlyPro) does not have hormonal activity, but on the other hand, it stimulates expression of BDNF (Brain-derived Neurotropic Factor), which showed to be a potent modulator of synaptic plasticity in the astrocytes [16].

Immunoenzymatic sandwich analysis showed that 3 hours after laboratory mouse were given ACTH 4-10 ProGlyPro with a dosage of 50-250 mcg/kgs of body weight, BDNF were significantly increased in the frontal lobe [17]. In ischemic or hypoxia state, ACTH 4-10 ProGlyPro also increases mitochondrial resistance against "Calcium Stress" [18]. Thus delay calcium dysregulation and lessen mitochondrial function in the granular cell of the cerebellum with glutamate neurotoxicity. Melanocortin has an anti-inflammatory effect, and because ACTH 4-10 ProGlyPro has a similar structure, it is expected to have the same effect on inflammation [19].

The primary goal in treating SCI is to prevent secondary lesions of inflammation, thus prevent further disability [20]. This research wanted to focus on ACTH 4-10 ProGlyPro's effect which acts as an anti-inflammation, and improvement of motoric function upon treatment with those drugs in an animal study.

To prove the anti-inflammation effect of ACTH 4-10 ProGlyPro, we will conduct an experimental study on Wistar mouse with a given SCI [21]. The hypothesis would be there are significant effects to prevent astrocytes damage, thus improve the motoric function of the experimented mouse. When it does, ACTH 4-10 ProGlyPro certainly will enhance the quality of life in SCI patients, by preventing the formation of a secondary lesion that inhibits neural repair.

2. Materials and Method

2.1. Collection of Samples

The study was carried out at Primate Research Center, Bogor Agricultural Institute, PT. Bimana Indomedical ACUC from March 2015 to December 2016.

This is an experimental study; Subjects included if fulfill the following criteria; Adult Wistar female mouse, White, Weighted 250-300 grams. Subjects drop out if meet the following criteria animals which died within the observation period, animals which got infected at the surgical sites or having sepsis, animals with no improvement or worsening damage due to twisting compression of the spinal cord (wrong compression). All mice which fulfilled the inclusion/drop out criteria were recruited as samples. Total samples for this study were 32 mice.

2.2. Study design

Real experimental by using 32 female Wistar mice weighing between 250-300 grams of white compressed on the marrow of spinal cord as high as Thoracic 9-11th with 26-gram force compression divided into three treatment groups (given ACTH 4-10 ProGlyPro) and I group Control (without ACTH 4-10 ProGlyPro). The treatment group consisted of 8 wistar mice each given ACTH 4-10 ProGlyPro, 1, 3 and 6 hours post-compression, BBB scale was examined at days 1, 7, 14, 21, and 28 post-compression.

2.3. Procedure

This experimental study was performed by damaging the spinal cord of the white female Wistar mouse. The spinal cord was opened at the level of T9 to T11th; then a 26 grams-clamp was used for one minute to make Moderate compression injury in the spinal cord. This mouse has an approximation weight between 250-300 grams each. Then the mouse was separated into four treatment groups, which three were given ACTH 4-10 ProGlyPro in 1 hour, 3 hours, and 6 hours after compression respectively; and one control group. First, the investigator divided the mouse into eight working batch, due to inability to operate them at once because of the time-consuming compression injury. Then the injury was given by open surgery to the spinal cord at the level of Thoracic vertebrae 9 to 11th with a 26 grams clamp compression for one minute. Each mouse was separated into four groups of treatment, with the dosage of ACTH 4-10 ProGlyPro is 250 mcg per kg of body weight. After the first treatment, the three groups were given ACTH 4-10 ProGlyPro, once a day continuously until the 28th day. We used ACTH 4-10 ProGlyPro with a concentration of 0.1%. Therefore the mouse got 1 Nasal drop per day because the duration of action was expected to be 20-24 hours. The motoric function of each mouse was observed using Basso, Beattie, Bresnahan scale at the 1st, 7th, 14th, 21st, and 28th day.

2.4. Statistical analysis

Data analysis used SPSS (Statistical Package for the Social Science) in affiliation with Medical Faculty of Universitas Hasanuddin, Indonesia with statistical $p < 0.05$. To calculate the differences of BBB scale between control and treatment groups, we compared means using ANOVA (analysis of variances) and LSD (Least Significant Difference). As for the ethic view in this animal study, it could only be conducted if it was a high standard purpose of research, the method designed to support our hypothesis, can't be achieved using alternative subject or procedure, and it has greater advantages than animal suffering. Therefore we used 3R principles: Replacement, Reduction, and Refinement.

2.5. Ethical Clearances

This study was performed following approval from PT. Bimana Indomedical's Animal Care and Use Committee (ACUC Protocol No. R.03-15-IR).

3. Results

Characterisation of subjects

Data obtained from motor function (BBB scale value) on observation 1st day, 7th day, 14th day, 21st day and 28th

day post compression on 32 rats; Each 8 for the control group (K, no ACTH 4-10 ProGlyPro), 8 for P1 group (received ACTH 4-10 ProGlyPro 1 hour post treatment), 8 for P2 group (received ACTH 4-10 ProGlyPro 3 hours post compression) and 8 head for P3 group (get ACTH 4-10 PGP 6 hours post compression). The analysis results are presented with systematic as follows:

The results of descriptive analysis

Summary of descriptive analysis of motor function (BBB scale) on each observation day for various treatment groups can be seen in Table 1.

Table 1: Mean, median and SD BBB scales in various groups and observations

| Group | Mean±SD(Median) BBB scale | | | | |
|---------|---------------------------|----------------|----------------|----------------|----------------|
| | Observation (day) | | | | |
| | Hari-1 | Hari-7 | Hari-14 | Hari-21 | Hari-28 |
| K (n=8) | 0,0±0,0(0,0) | 4,1±2,9(3,5) | 7,0±3,2(7,0) | 9,9±2,4(9,0) | 12,0±2,7(11,0) |
| P1(n=8) | 2,0±3,9(0,0) | 9,5±2,3(8,0) | 14,5±2,4(14,5) | 18,4±1,9(19,0) | 20,5±1,1(21,0) |
| P2(n=8) | 2,4±2,6(2,0) | 10,0±2,9(10,5) | 14,6±3,7(16,0) | 17,8±2,3(18,0) | 20,0±1,4(20,5) |
| P3(n=8) | 1,3±2,4(0,0) | 9,9±2,5(10,5) | 14,0±1,9(14,0) | 17,5±2,3(18,0) | 19,8±2,1(21,0) |

K = control (not getting ACTH 4-10 ProGlyPro; P1 = received ACTH 4-10 ProGlyPro 1 hour post compression; P2 = ACTH 4-10 ProGlyPro 3 hours post compression; P3 = ACTH 4-10 ProGlyPro 6 hours post compression

From Table 1 above it can be seen that in the treatment group and did not get the ACTH 4-10 ProGlyPro (control group) had mean and standard intersection (mean ± SD) BBB scale $0,0 \pm 0,0$ on day-1; Increased to 4.1 ± 2.9 on day-7, to 7.0 ± 3.2 on day-14, to 9.9 ± 2.4 on day-21 and to 12.0 ± 2.7 days -28. Thus, although not given ACTH 4-10 ProGlyPro, there will be an improvement in motor function (BBB scale) as the recovery time increases. The longer the recovery time (observation day), the higher the BBB scale. The same is true for groups given ACTH 4-10 PGP post compression; With a higher BBB scale. All groups (P1, P2, and P3) had a higher BBB scale than the BBB-scale control group, at least 2-fold at each recovery time (1st day, 7th day, 14th day, 21st day, and 28th day) post compression. However, there was no difference in BBB scale between groups receiving ACTH4-10PGP, although the time of administration post compression was different (1 hour, 3 hours and 6 hours post compression).

From figure 1 it can be seen that the mean BBB scale in each cluster has increased along with the increase of observation time (recovery) post compression. The longer the recovery time, the higher the BBB scale mean in all groups including the control group (K), but the group receiving ACTH 4-10 ProGlyPro (P1, P2, and P3) shows the figure above the control group figure; And the three coincide with each other); Even if the P1 group is located above the group P2 and P3; Especially on the 14th day, 21st day and 28th day observations.

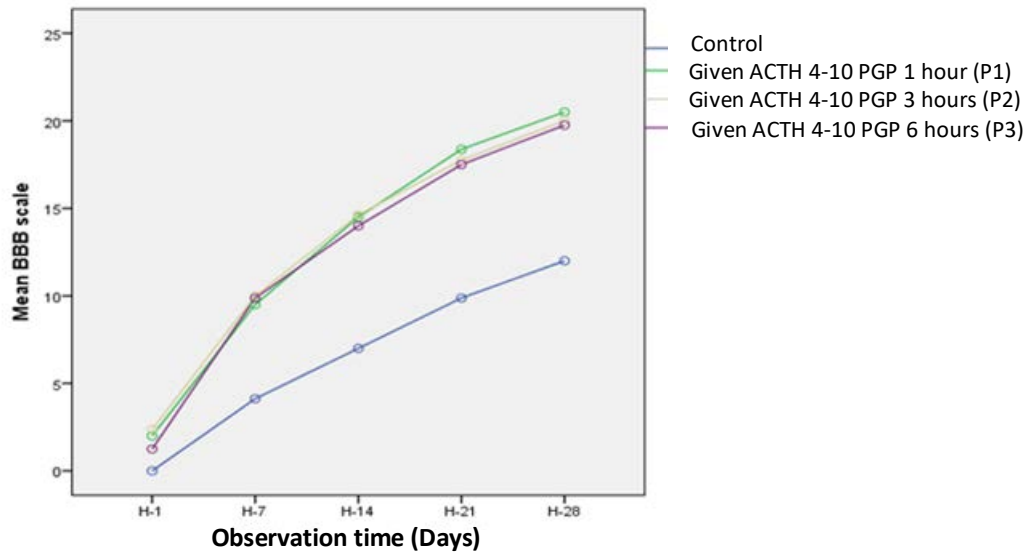


Figure 1: Mean BBB scale of each group at various observation times (recovery)

Effects of ACTH 4-10 ProGlyPro administration on motor function recovery post compression

To assess the effect of ACTH 4-10 ProGlyPro administration on motor function recovery post compression and the effect of post compression, One-Way ANOVA, and LSD tests were performed on the 7th day, 14th day, 21st day and 28th day BBB scales Post compression in each group. Summary of the results can be seen in Table 2.

Table 2: Comparison of BBB scale increase by group

| Group | Mean±SD(Median) Increased BBB scale | | | |
|---------|-------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Observation time (day) | | | |
| | H7-H1 | H14-H1 | H21-H1 | H28-H1 |
| K (n=8) | 4,1±2,9(3,5) ^a | 7,0±3,2(7,0) ^b | 9,9±2,4(9,0) ^b | 12,0±2,7(11,0) ^c |
| P1(n=8) | 7,5±3,2(8,0) ^b | 12,5±3,1(13,5) ^c | 16,4±4,4(18,5) ^d | 18,5±3,9(21,0) ^d |
| P2(n=8) | 7,6±2,3(8,0) ^b | 12,3±2,7(12,0) ^c | 15,4±2,1(14,5) ^d | 17,6±2,1(17,0) ^d |
| P3(n=8) | 8,6±3,5(9,0) ^b | 12,8±3,1(13,5) ^c | 16,3±3,8(18,0) ^d | 18,5±3,3(20,5) ^d |

One Way ANOVA and LSD; The same superscript in the same column showed no significant difference ($p > 0.05$); And if different means different meaning ($p < 0.05$).

From Table 2 it can be seen that on 7th day post compression there was an increase in BBB scale with mean of 4.1 in the control group, and increased higher in the group given ACTH 4-10 ProGlyPro; that is as big as 7.5 in group P1, equal to 7.6 in group P2, and equal to 8.6 in group P3. Similarly on 14th day (7.0 vs 12.5 vs. 12.3 vs 12.8), on 21st day (9.9 vs 16.4 vs 15.4 vs 16.3), on 28th day (12.0 vs 18.5 vs. 17.6 vs 18.5).

This suggests that administration of ACTH 4-10 PGP post compression increases the BBB scale (higher motor function) greater than that without ACTH 4-10 ProGlyPro post compression at the same time. In other words, the provision of ACTH 4-10 ProGlyPro accelerates the recovery of motor function post compression. The effect of time difference of ACTH 4-10 ProGlyPro (P1, P2, and P3) was not available because there was no significant ($p > 0.05$) between P1, P2, and P3 at each observation time.

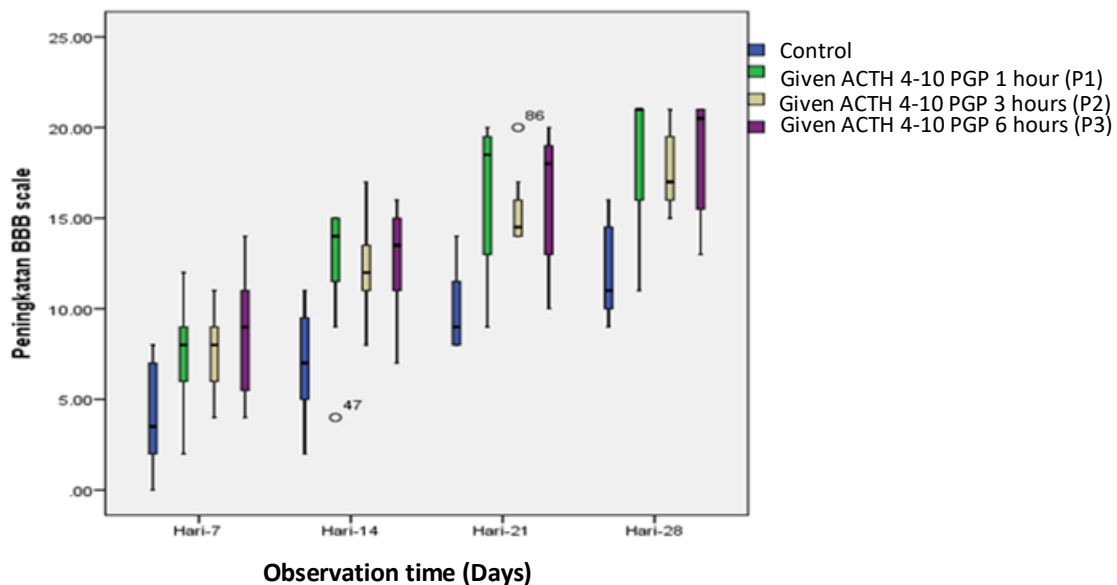


Figure 2: Box plot of increase in BBB scale between groups on observations 7th day, 14th day, 21st day and 28th day.

From the figure above can be seen that there is an increase BBB scale, where the increase of BBB scale (amount of motor recovery) depends on two things that are observation time (recovery) and group (giving ACTH 4-10 ProGlyPro). The longer the recovery time, the greater the increase in BBB scale (the higher the recovery of motor function). Giving ACTH 4-10 ProGlyPro post compression had a higher BBB scale increase than control (not given ACTH 4-10 ProGlyPro) at each observation time, indicating that administration of ACTH 4-10 PGP post compression accelerated the recovery of motor function. The time difference of ACTH 4-10 ProGlyPro post compression did not result in differences in motor recovery acceleration. Limitations of this study were inadequate clamping tools that may lead to different clasps on the right and left sides.

4. Discussion

During our observation, it was a challenge to assess BBB scale of the mouse with asymmetric motoric function. For the purpose of the study, we take the best motoric scale between the two legs. For the future consideration, it may be best to choose different scale to assess the motoric function of a mouse with spinal cord injury, such as Neurological Severity Scores. In the previous research, ACTH 4-10 ProGlyPro had been used for treatment and prevention of brain injury complication. Though it is still a long way, ACTH 4-10 ProGlyPro could be one of option in the treatment of spinal cord injury too. Therefore, further research to determine optimal dosage, minimal functional dosage, lowest lethal dosage, and other pharmacological study in human are highly

encouraged [15, 23, 27]. In human, the degree of spinal cord injury, or degree of its disability, were determined using ASIA scale. With this scale, we could divide the injury into mild, moderate, or severe form. The outcome would affect the patients' mental state, direct as well as indirect cost, and his/her quality of life. This research has not determined the degree of SCI that's given to the mouse. Thus another experimental study with different clamp weight to generate mild, moderate, or severe SCI, is also recommended [25,26]. The timing of ACTH 4-10 ProGlyPro treatment was chosen based on the timing of SCI patients being brought to the emergency hospital. The fastest was one hour, some within three hours, and others are brought in at least six hours after SCI. This study was trying to determine golden hours in the treatment of ACTH 4-10 ProGlyPro in SCI. Unfortunately, the result was not statistically significant. The treatment of this drug as anti-inflammatory was believed to prevent the formation of a secondary lesion that can interfere with the neural repair. The secondary lesion is formed within 24 hours after the initial injury. But many factors influence this process, including hypoxia and ischemia, oxidative stress, and other inflammatory contribution. The condition of these factors could differ between human and other mammals or mouse in this case. This could cause the insignificance of the result between the treatment groups [15,16,23, 27].

5. Conclusion

Postoperative ACTH 4-10 PGP post compression can accelerate recovery of motor function post compression. There was a tendency of motor function recovery effects on post compression of ACTH 4-10 ProGlyPro better on 1-hour post compression, but not statistically significant. Postoperative ACTH 4-10 ProGlyPro post compression affects the level of spinal cord tissue damage following compression. Postoperative ACTH 4-10 PGP administration reduced the extent of cell damage to the spinal tissue of the spinal cord post compression. There is a difference in the rate of damage of spinal cord tissue post compression based on the difference of time of administration of ACTH 4-10 PGP post compression. The faster the ACTH 4-10 ProGlyPro is given post compression, the lower the level of cell damage of the spinal tissue astrocytes of the spinal cord. Administration of ACTH 4-10 PGP 1 hour post compression was best compared with 3 and 6 hours post compression. Suggested for further study using different staples to compare mild, moderate, and severe spinal cord injury. Improvement of the clamping device resulting in a uniform spinal cord injury so that the right and left sections of the spinal cord. To get the same pressure or compression at the time of clamping so that the right and left legs occur in the same paralysis and are expected to happen in both legs during the study And result calculation.

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6. Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare

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