

# Effect of ACTH4-10pro8-gly9-pro10 on malondialdehyde and F2-isoprostane 15(S)-8-iso-PGF2a expression in rat spinal cord injury

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## Effect of ACTH4-10pro8-gly9-pro10 on malondialdehyde and F2-isoprostane 15(S)-8-iso-PGF2a expression in rat spinal cord injury

Efecto del ACTH4-10pro8-gly9-pro10 en la expresión de malondialdehído y F2-isoprostano 15(S)-8-iso-PGF2a en ratas con lesión de médula espinal

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### SUMMARY

**Background:** Spinal cord injury (SCI) is damage to the spinal cord caused mainly by trauma resulting in major motor, sensory, and autonomic dysfunctions. This experimental study aims to assess the effect of ACTH4-10Pro8-Gly9-Pro10 administration in rats with mild and severe compression injuries on the levels of malondialdehyde and F2-isoprostane 15(S)-8-iso-PGF2a levels.

**Methods:** Mild and severe spinal cord compression was performed on 27 subjects. They were divided into three groups of samples with control included, 3 and 6 h after the injury. Spinal cord tissue was taken to

determine MDA and F2-isoprostane expression under the microscope, and control samples were compared to samples given ACTH4-10Pro8-Gly9-Pro10.

**Results:** In 3- and 6-h-mild ASCI that ACTH<sub>4-10</sub>Pro<sup>8</sup>-Gly<sup>9</sup>-Pro<sup>10</sup> is given, MDA expression was lower ( $8.40 \pm 1.94$  and  $8.60 \pm 1.67$ ) than the control group ( $12.60 \pm 2.6$  and  $14.40 \pm 1.81$ ). Meanwhile, severe ASCI that ACTH<sub>4-10</sub>Pro<sup>8</sup>-Gly<sup>9</sup>-Pro<sup>10</sup> also exhibited lower MDA expression ( $9.8 \pm 2.16$  and  $12.2 \pm 1.92$ ) than the control group ( $16.2 \pm 1.6$  and  $16.40 \pm 2.07$ ). This is in line with the lower expression of F2-isoprostane in acute myelin injuries with mild ( $5.0 \pm 2.0$  and  $7.6 \pm 1.34$ ) and severe ( $8.0 \pm 2.23$  and  $11.8 \pm 1.84$ ) compression.  $1.64$ ) compared to controls (mild:  $12.8 \pm 3.03$  and  $13.8 \pm 2.58$ ; severe:  $15.4 \pm 2.60$  and  $16.4 \pm 2.30$ ) at 3- and 6-hours post-wound.

**Conclusion:** Administration of ACTH<sub>4-10</sub>Pro<sup>8</sup>-Gly<sup>9</sup>-Pro<sup>10</sup> decreased MDA and F2 isoprostane expression in mild and severe spinal cord compression models. Thus, it might become adjuvant therapy for spinal cord injury to prevent further secondary injury.

**Keywords:** ACTH<sub>4-10</sub>Pro<sup>8</sup>-Gly<sup>9</sup>-Pro<sup>10</sup>, spinal cord injury, reactive oxygen species, malondialdehyde, F2-isoprostane.

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## RESUMEN

**Antecedentes:** Lesión de la médula espinal es un daño a la médula espinal causado principalmente por traumatismos que resultan en disfunciones motoras, sensoriales y autónomas importantes. Este estudio experimental tiene como objetivo evaluar el efecto de la administración de ACTH4-10Pro8-Gly9-Pro10 en ratas con lesiones por compresión leves y graves en los niveles de malondialdehído y los niveles de F2-isoprostanos 15(S)-8-iso-PGF2 $\alpha$ .

**Métodos:** Se realizaron compresiones medulares leves y graves en 27 sujetos, que luego fueron divididos en tres grupos de muestras con control incluido, 3 y 6 horas tras la lesión. Se tomaron muestras de tejido medular para determinar la expresión de MDA y F2-isoprostano en el microscopio, y se compararon con muestras de control a las que se había suministrado ACTH410Pro8-Gly9-Pro10.

**Resultados:** En las muestras de lesiones leves 3 y 6 horas a las que se había administrado ACTH<sub>4-10</sub>Pro<sup>8</sup>-Gly<sup>9</sup>-Pro<sup>10</sup>, la expresión de MDA fue menor ( $8,40 \pm 1,94$  and  $8,60 \pm 1,67$ ) que en el grupo de control ( $12,60 \pm 2,6$  and  $14,40 \pm 1,81$ ). Por otro lado, en las lesiones graves con ACTH<sub>4-10</sub>Pro<sup>8</sup>-Gly<sup>9</sup>-Pro<sup>10</sup> también se halló una expresión de MDA menor ( $9,8 \pm 2,16$  and  $12,2 \pm 1,92$ ) que en el grupo de control ( $16,2 \pm 1,6$  and  $16,40 \pm 2,07$ ). Esto es coherente con la expresión menor de F2isoprostano en las lesiones agudas de mielina con compresión leve ( $5,0 \pm 2,0$  and  $7,6 \pm 1,34$ ) y grave ( $8,0 \pm 2,23$  and  $11,8 \pm 1,84$ ) comparada con las muestras de control (leve:  $12,8 \pm 3,03$  y  $13,8 \pm 2,58$ ; grave:  $15,4 \pm 2,60$  y  $16,4 \pm 2,30$ ) 3 y 6 horas tras la lesión.

**Conclusión:** La administración de ACTH4-10Pro8-Gly9-Pro10 disminuyó la expresión de MDA y los isoprostanos F2 en el modelo de compresión de la médula espinal leve y grave. Por lo tanto, podría convertirse en una terapia coadyuvante para lesiones de la médula espinal, con el fin de prevenir lesiones secundarias adicionales.

**Palabras clave:** ACTH<sub>4-10</sub>Pro<sup>8</sup>-Gly<sup>9</sup>-Pro<sup>10</sup>, lesión medular, especies reactivas del oxígeno, malondialdehído, F2-isoprostano.

## INTRODUCTION

Spinal cord injury (SCI), also known as myelin injury, is a medical condition with a high morbidity rate due to the limited capacity of central axon regeneration. This limitation arises from the distinctive properties of glial cells in the central nervous system (CNS), preventing them from adapting to an environment conducive to

localized healing (1). Myelin injury represents a significant contributor to human morbidity, presenting a formidable challenge in terms of treatment. When examining the progress achieved in terms of outcomes, it becomes apparent that advancements in palliative care and functional recovery have been relatively stagnant in comparison to other significant developments witnessed in the history of modern medicine (2).

In the United States, more than one million individuals experience paralysis because of spinal cord injuries. Among healthy individuals aged 19 to 29 years, acute spinal cord injury (ASCI) represents the most prevalent type of injury, leading to many patients suffering from significantly diminished quality of life for extended periods following the injury (3). According to data from Fatmawati General Hospital, Jakarta, Indonesia in 2014, out of 104 cases, 37 were attributable to traumatic causes, while 67 were non-traumatic in nature (4). Similarly, data obtained from Dr. Soetomo General Academic Hospital in Surabaya for the period 2013-2017 revealed 442 patients with spinal fractures, with a higher incidence among males compared to females, with a ratio of 3.3:1 (5).

Since the discovery of antibiotics, the prevention of complications, and the introduction of unique treatment methods, developments in the treatment of spinal injury patients have made significant progress (6). Several studies of spinal injuries in mammals show that injury recovery is supported by compensatory anatomical plasticity of the entire nervous system, so the survival rate of spinal injury patients has increased rapidly. However, to date, no effective therapy that improves sufferers' neurological and functional conditions is reported (7).

The development of surgical techniques has made it possible to decompress the spinal nerves, restore normal spinal curvature, and stabilize and/or fusion. The primary objective of these interventions is to alleviate pain, promote early mobilization and rehabilitation, minimize the duration of treatment, and prevent secondary complications associated with disability (8).

ACTH4-10 (ACTH4-10Pro8-Gly9-Pro10) is a neuroprotective agent commonly used in stroke, head injury, and Alzheimer's patients. Its

structure is similar to melanocortin, which can bind to melanocortin receptors and provide anti-inflammatory effects. To date, no data or reports regarding serious side effects from its use (9). ACTH4-10 administration is known to reduce the apoptosis rate of neuron cells in acute spinal cord compression injury in Sprague-Dawley rats by increasing the Bcl-2/HSP70 ratio (10). Other studies show that giving ACTH4-10 can also reduce pro-inflammatory mediators TLR-2, NF- $\kappa$ B, IL-8, and TNF- $\alpha$  in Sprague-Dawley rats treated with acute spinal cord compression injury (11).

Spinal cord injury pathology is influenced by an intricate cascade process, one of the most significant is oxidative stress, which is caused by the formation of free radicals and lipid peroxidation (12). Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes the overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress (13). F2-isoprostane 8-iso-PGF<sub>2 $\alpha$</sub>  is a thromboxane A<sub>2</sub> (TXA<sub>2</sub>) receptor agonist, which is a stable biomarker of oxidative stress (14). Therefore, understanding ACTH4-10Pro8-Gly9-Pro10's influence to reduce oxidative stress markers, such as malondialdehyde (MDA) and F2-isoprostane, could provide valuable insights into potential therapeutic strategies for spinal cord injury (15).

This study used Sprague-Dawley rats as a spinal injury model, clamped with clips to produce light and heavy compression. The experimental animals were then given intranasal ACTH4-10Pro8-Gly9-Pro10. Myelin was taken for immunohistochemical examination to identify malondialdehyde (MDA) and F2-Isoprostan 15(S)-8-iso-PGF<sub>2 $\alpha$</sub>  to determine its effect.

## MATERIALS AND METHODS

### Animals

Twenty-seven white male Sprague-Dawley rats aged 12 weeks (250-300 g) were randomly selected and divided into the control and treatment groups. The rats were acclimatized for two weeks for adaptation in the rearing facility. The rat food was given in the form of pellets. Food and drink for experimental animals were given ad

libitum, and the cage temperature was maintained at around 25°C, with dark-light cycle every 12 h. Each group of rats was placed in a separate cage and kept so that they did not interact with each other.

Anesthesia was carried out by administering ketamine combined with xylazine. Ketamine was injected intraperitoneally using a 10 mL syringe containing 10 mL of ketamine (100 mg/mL) and 1 mL of xylazine (100 mg/mL). The drug mixture was injected as much as 0.1 mL/100 g of rat body weight. The drug mixture contains 91 mg/kg of ketamine and 9.1 mg/kg of xylazine. The loss of the carpedal reflex served as an indicator that the anesthetic agent had been adequately administered, signaling that the surgical procedure could commence immediately. The duration of the anesthetic agent's effect was approximately 60-80 min. Following the injection, the hemodynamic function was monitored. This included observing the mucous membranes, which should appear pink and moist, as well as assessing the capillary refill time, which should be less than 2 seconds (16).

For the compression procedure, modified aneurysm clips were employed as compression devices, calibrated to exert clamping forces of 20 g and 35 g. After performing a laminectomy and exposing the dura, the aneurysm clip was affixed to the myelin. Compression was applied for 1 minute, then released and closed again using sutures (17).

### Fabrication of tissue block paraffin

The myelin tissue underwent an initial washing step with PBS (Phosphate Buffered Saline) for 3-5 cycles to eliminate contaminants. Subsequently, the tissue was fixed in 10 % formalin. Dehydration of the tissue was then carried out using a series of graded ethanol solutions, where each concentration (30 %, 50 %, 70 %, 80 %, 96 %, and absolute) was applied for 60 minutes. Following dehydration, a clearing process was performed, involving two immersions of 60 min each in xylene. Subsequent infiltration was achieved by subjecting the tissue to soft paraffin at a temperature of 48°C for 60 min. A block was then created using hard paraffin in a mold, allowing it to solidify over a day. On the following



day, the block was sectioned into slices measuring 4-6  $\mu\text{m}$  in thickness using a rotary microtome. These sections were subsequently mounted on glass slides using 5 % gelatin. To process the slides obtained from the paraffin block, they were immersed in xylene twice, with each immersion lasting 5 min. Subsequently, rehydration was carried out by exposing the slides to a series of sequential alcohol solutions (absolute, 96 %, 80 %, 70 %, 50 %, and 30 %) for 5 min each. Finally, the slides were rinsed in  $\text{H}_2\text{O}$  for 5 min.

#### Determination of MDA and F2-isoprostane expression

The slides underwent a 5-min washing step with PBS pH 7.4. The blocking procedure was performed using 3 %  $\text{H}_2\text{O}_2$  for 20 min. The subsequent washing procedure consisted of three repetitions, each lasting 5 min, employing PBS pH 7.4. A 5 % FBS and 0.25 % Triton X-100 solution were employed for protein unspecific blocking. The washing process was repeated three times, each lasting 5 min, employing PBS pH 7.4. Incubation with anti-MDA and anti-F2 isoprostane polyclonal rabbit antibody was conducted for 60 minutes. The washing process, consisting of three repetitions lasting 5 minutes each, was carried out using PBS pH 7.4. Subsequently, the slides were treated with Diaminobenzidine (DAB), incubated for 10 minutes, and subjected to a three-time washing process, each lasting 5 minutes, utilizing PBS pH 7.4. Counterstaining was performed using Mayer Hematoxylin, with a set duration of 10 minutes, followed by washing with tap water,

rinsing with  $\text{H}_2\text{O}$ , air-drying, and subsequent observation under a light microscope.

#### Data Analysis

Data normality analysis was performed using Kolmogorov-Smirnov. Data on levels of MDA and F2-Isoprostane were analyzed using the Paired T-test and ANOVA.

## RESULTS

#### MDA Expression on Paraffin Block IHC Staining Animal Model of Acute Spinal Cord Compression Injury

In this study, a total of three samples were collected from each control group and treatment group, specifically observing the effects of distilled water and ACTH4-10Pro8-Gly9-Pro10 at 3 and 6 h. The samples were obtained from rats subjected to both light and heavy compression injuries, aiming to evaluate the expression of immunoreactivity to MDA staining, which serves as an indicator of the response to ACTH4-10 in the spinal cord injury (SCI) model. To assess the effects of ACTH4-10, cross-sections of the rat spinal cord were obtained from rats treated with mild acute spinal cord compression injury (ASCI). These sections were then dripped with 0.9 % NaCl solution and sacrificed after 3 hours (K+1.1). Subsequently, the sections were examined under a microscope at a magnification of 400x.

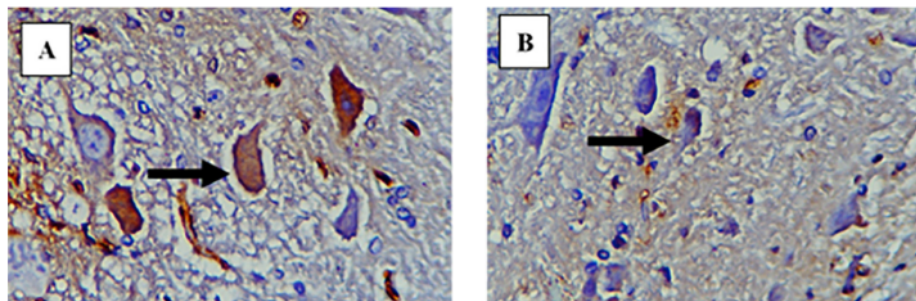


Figure 1. Cross-section of the spinal cord of rats treated with mild ASCI and sacrificed after 3 hours of staining with IHC MDA (A) dripped with 0.9 % NaCl (B) dripped with ACTH4-10Pro8-Gly9-Pro10

Positive neuronal responses expressing MDA were identified using black arrows (Figure 1. A). It was observed that the preparation dripped with

ACTH4-10Pro8-Gly9-Pro10 drops significantly reduced the response of neurons expressing MDA (Figure 1. B).

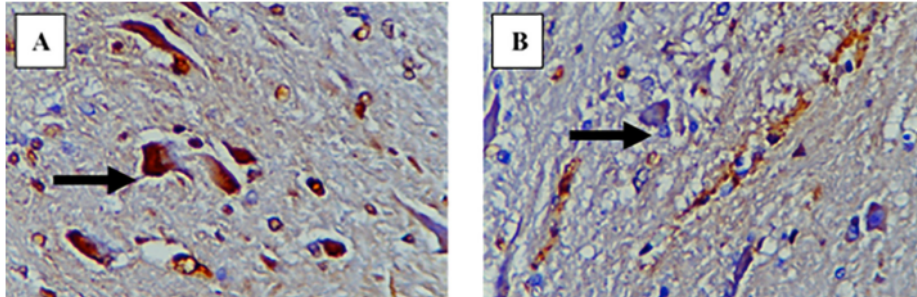


Figure 2. Cross-section of the spinal cord of rats treated with severe ASCI and sacrificed after 6 hours of staining with IHC MDA (A) dripped with 0.9 % NaCl (B) dripped with ACTH4-10Pro8-Gly9-Pro10.

Figure 2A illustrates a cross-section of the spinal cord from rats subjected to severe acute spinal cord injury. The section was treated with 0.9 % NaCl solution and sacrificed after 6 h. The image was captured using a microscope at a magnification of 400x. Positive neuronal

responses expressing MDA are indicated by black arrows. In Figure 2B, it can be observed that the response of neurons expressing MDA was significantly reduced when the preparation with ACTH4-10Pro8-Gly9-Pro10 drops was administered.

### F2-Isoprostane Expression on Paraffin Block IHC Staining Animal Model of Acute Spinal Cord Compression Injury

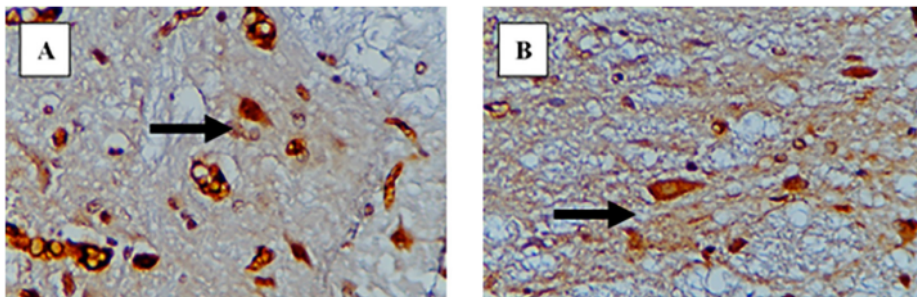


Figure 3. Cross-section of the spinal cord of rats treated with mild ASCI and sacrificed after 3 h of staining with IHC F2 Isoprostane (A) dripped with 0.9 % NaCl (B) dripped with ACTH4-10Pro8-Gly9-Pro10

Figure 3A presents a cross-section of the spinal cord from rats treated with mild acute spinal cord injury. The section was dripped with 0.9 % NaCl solution and sacrificed after 3

hours (K+1.1). The image was captured using a microscope at a magnification of 400x. Positive neuronal responses expressing F2-isoprostane are indicated by black arrows. In Figure 3B, it

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can be observed that the preparation given with ACTH4-10Pro8-Gly9-Pro10 drops significantly

reduced the response of neurons expressing F2-isoprostane.

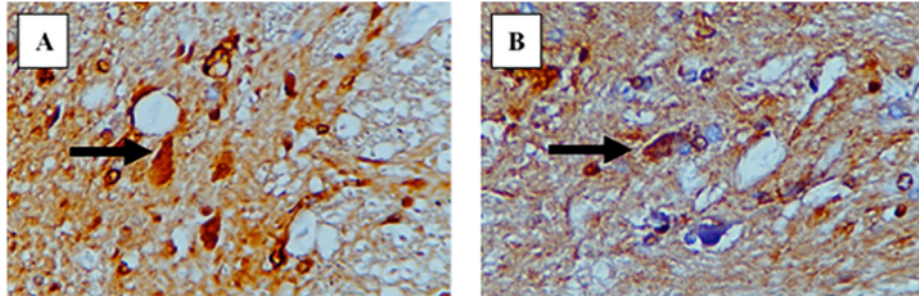


Figure 4. Cross-section of the spinal cord of rats treated with severe ASCI and sacrificed after 6 hours of staining with IHC F2 Isoprostane (A) dripped with 0.9 % NaCl (B) dripped with ACTH4-10Pro8-Gly9-Pro10.

Figure 4. A illustrates a cross-section of the spinal cord from rats subjected to severe acute spinal cord injury. The section was treated with 0.9 % NaCl solution and sacrificed after 6 h. The image was captured using a microscope at a magnification of 400x. Positive neuronal

responses expressing F2-isoprostane are indicated by black arrows. Similarly, in Figure 4. B it can be observed that the preparation with ACTH4-10Pro8-Gly9-Pro10 drops led to a significant reduction in the response of neurons expressing F2-isoprostane.

**The Results of The Expression of MDA and F2-isoprostane in Each Group**

Table 1. MDA Expression in The Mild and Severe ASCI Group

Group	MDA Expression		P
	Mean	Standard deviation	
Mild ASCI NaCl 0.9 % 3 h	12.60	2.60	-
Mild ASCI NaCl 0.9 % 6 h	14.40	2.45	-
Mild ASCI ACTH4-10Pro8-Gly9-Pro10 3 h	8.40	1.4	0.022
Mild ASCI ACTH4-10Pro8-Gly9-Pro10 6 h	8.60	1.67	0.003
Severe ASCI NaCl 0.9 % 3 h	16.20	2.16	-
Severe ASCI NaCl 0.9 % 6 h	16.40	2.07	-
Severe ASCI ACTH4-10Pro8-Gly9-Pro10 3 h	9.80	2.16	0.002
Severe ASCI ACTH4-10Pro8-Gly9-Pro10 6 h	12.20	1.92	0.011

Regarding the expression of MDA in each respective group, it was observed that the group receiving ACTH4-10Pro8-Gly9-Pro10 and sacrificed at 3 and 6 h exhibited a significant

reduction in the mean value compared to the group that did not receive ACTH4-10Pro8-Gly9-Pro10 (Table 1).



Table 2. F2 Isoprostane Expression in The Mild and Severe ASCI Group

Group	F2 Isoprostane Expression		
	Mean	Standard deviation	P
Mild ASCI NaCl 0.9% 3 hours	12.80	3.03	-
Mild ASCI NaCl 0.9% 6 hours	13.80	2.58	-
Mild ASCI ACTH4-10Pro8-Gly9-Pro10 3 h	5.0	2.0	0.002
Mild ASCI ACTH4-10Pro8-Gly9-Pro10 6 h	7.60	1.34	0.003
Severe ASCI NaCl 0.9% 3 h	15.40	2.60	-
Severe ASCI NaCl 0.9% 6 h	16.40	2.30	-
Severe ASCI ACTH4-10Pro8-Gly9-Pro10 3 h	8.0	2.23	0.001
Severe ASCI ACTH4-10Pro8-Gly9-Pro10 6 h	11.80	1.64	0.008

Likewise, in terms of F2-isoprostane expression, the group subjected to ACTH4-10Pro8-Gly9-Pro10 administration and sacrificed at 3 and 6 h demonstrated a notable decrease in the mean value in comparison to the group without ACTH4-10Pro8-Gly9-Pro10 administration (Table 2).

### DISCUSSION

The findings of this study revealed that rat with mild acute spinal cord injury (ASCI), who received ACTH4-10Pro8-Gly9-Pro10 and were sacrificed at 3 h and 6 h post-treatment, exhibited a decreased expression of MDA compared to the group treated with 0.9 % NaCl. Specifically, in the treatment group of mild ASCI rats administered with ACTH4-10Pro8-Gly9-Pro10, the MDA expression was measured at 8.60 (1.67), which was significantly lower than the group treated with 0.9 % NaCl, measuring 12.60 (2.60). These results were statistically significant between the two groups, with p-values after 3 h and 6 h being 0.022 and 0.003, respectively.

In the severe ASCI treatment group given ACTH4-10Pro8-Gly9-Pro10 and sacrificed after 3 and 6 h, the MDA expressions were 9.80 (2.16) and 12.20 (1.92), respectively, lower than the group given 0.9 % NaCl, namely 16.20 (2.16) and 16.40 (2.07). These results were also statistically significant, with p values after 3 h and 6 h of 0.002 and 0.011. From the above data, the mild and severe ASCI given ACTH4-10Pro8-Gly9-Pro10

showed a decrease in the expression of MDA, which was lower and statistically significant compared to the group given 0.9 % NaCl. This finding supports Puspita et al., who reported that administration of ACTH4-10Pro8-Gly9-Pro10 could reduce MDA levels from 86.31 % from  $3.11 \pm 0.028 \mu\text{g/mL}$  to 40.46 % from  $1.85 \pm 0.020 \mu\text{g/mL}$ . This is possibly accounted for by the transformation mechanism of the metabolic chain that reduces inflammatory factors and increases anti-inflammatory factors. Where ACTH4-10 peptides induce this mechanism, this reaction also reduces lipid peroxidation. ACTH4-10 peptides act as antioxidants that inhibit the formation of free radicals and prevent or inhibit lipid peroxidation. The hydroxyl group (OH) on the ACTH4-10 peptide may serve as an antioxidant. The mechanism of inhibition of free radicals by ACTH4-10 is by covering the hydroxyl groups and donating hydrogen atoms to lipid radicals. ACTH4-10 peptide suppresses the activity of the lipoxygenase enzyme, resulting in damage due to oxidative stress. The radicals from ACTH4-10 peptides are relatively more stable than lipid radicals, so the radicals from ACTH4-10 peptides do not have enough energy to react with other lipid molecules to form new lipid radicals. Thus, the ACTH4-10 peptide can reduce MDA levels (18).

The F2-isoprostane 8-iso-PGF<sub>2α</sub>, a TXA2 agonist is a stable biomarker of oxidative stress. In the mild ASCI treatment group given ACTH4-10Pro8-Gly9-Pro10 and sacrificed after 3 and 6 h, the expression of F2-isoprostane was lower compared to the group treated with



0.9 % NaCl. The expression of F2-isoprostane in the ACTH4-10Pro8-Gly9-Pro10 group was 5.0 (2.0), whereas, in the 0.9 % NaCl group, it was 12.80 (3.03). These differences in F2-isoprostane expression between the two groups were statistically significant, with p-values of 0.002 and 0.003 after 3 h and 6 h, respectively.

In the severe ASCI treatment group given ACTH4-10Pro8-Gly9-Pro10, the expression of F2-isoprostane was lower compared to the group treated with 0.9 % NaCl. After 3 h and 6 h, the expression of F2-isoprostane in the ACTH4-10Pro8-Gly9-Pro10 group was 8.0 (2.23) and 11.80 (1.64), respectively, while in the 0.9 % NaCl group, it was 15.40 (2.60) and 16.40 (2.30). From the above data, the mild and severe ASCI given ACTH4-10Pro8-Gly9-Pro10 showed a decreased F2-isoprostane expression, which was lower and statistically significant compared to the group given 0.9 % NaCl. The findings from our study support the previous research by Erny et al., which demonstrated that ACTH4-10 administration can reduce the level of prostaglandin E2 associated with NFκB caused by transcription factors (19).

In previous studies, ACTH4-10Pro8-Gly9-Pro10 has been utilized for the treatment and prevention of brain injury complications (20). Although ACTH4-10Pro8-Gly9-Pro10 application in spinal cord injury (SCI) remains limited, it holds promising potential as a therapeutic intervention. The timing of ACTH4-10Pro8-Gly9-Pro10 administration was selected within the initial 3 hours following SCI. This decision is based on considerations from glial cell cultures derived from newborn rat brains, which demonstrated that ACTH4-10Pro8-Gly9-Pro10 led to a rapid reduction in MDA and F2-isoprostane mRNA levels within a maximum duration of 40-60 minutes. Therefore, it can be inferred that alterations in protein levels are likely to be evident within a short duration of approximately 3 hours following the administration of ACTH4-10Pro8-Gly9-Pro10. Furthermore, the main goal of this study is to determine the optimal time window for administering ACTH4-10Pro8-Gly9-Pro10 in spinal cord injury (SCI) cases. The primary objective of administering ACTH4-10Pro8-Gly9-Pro10 is to alleviate secondary injury processes that hinder nerve regeneration, typically occurring within 24 h of the primary injury. Numerous factors influence the development of secondary

injury, including hypoxia, ischemia, oxidative stress, and inflammatory mediators. It is worth noting that the characteristics of these factors may differ between humans and other mammals. In this study, rats were used. Furthermore, the clinical administration of ACTH4-10Pro8-Gly9-Pro10 serves as a neuroprotective agent with the potential to modulate neurotransmitters, thereby inhibiting apoptosis and stimulating the production of neuronal protective substrates. In Filipenkov et al. study that used transient occlusion of the middle cerebral artery (MCA), administration of ACTH4-10Pro8-Gly9-Pro10 after 24 h after the injury affected 394 differentially expressed genes (DEGs), both upregulated and downregulated genes, specifically indicating a suppressive effect on inflammatory genes and roles in activating neurotransmitters and initiating the expression of mRNAs that are naturally impaired in ischemia (21).

In another study conducted by Loe et al. in 2020 using a brain injury model, ACTH4-10 Pro8-Gly9-Pro10 was reported to increase neuronal progenitor stem cells (NPSCs) in the subgranular zone of the hippocampal dentate gyrus (20). The results of this study can be used as the basis for further research on the effect of ACTH4-10 Pro8-Gly9-Pro10 administration on stem cell expression levels in models of acute myelin compression injury.

## CONCLUSIONS

Administration of ACTH4-10Pro8-Gly9-Pro10 decreases MDA and F2-isoprostane expression in mild and severe spinal cord compression models. Thus, it might become adjuvant therapy for spinal cord injury to prevent further secondary injury.

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