American Journal of Medical and Biological Research, 2017, Vol. 5, No. 2, 18-22
Available online at http://pubs.sciepub.com/ajmbr/5/2/2

DOI:10.12691/ajmbr-5-2-2

Downregulation of MMP-9 Level and GCS Score Improvement in Severe Traumatic Brain Injury Due to the Mild Hypothermia Therapy

Eko Prasetyo[1,*], Andi Asadul Islam[2], Mochammad Hatta[3], Djoko Widodo[2], Ilhamjaya Pattelongi[4]

[1]Departement of Surgery, Division of Neurosurgery, Faculty of Medicine, University of Sam Ratulangi, Manado, Indonesia
[2]Departement of Surgery, Division of Neurosurgery, Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia
[3]Molecular Biology and Immunology Laboratory, Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia
[4]Departement of Physiology, Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia
*Corresponding author: ekoprasetyo@unsrat.ac.id

Abstract We recently investigated the effect of the mild hypothermia therapy towards alteration of matrix metalloproteinase-9 (MMP-9) and outcome of severe traumatic brain injury (TBI). The neurologic outcome was assessed by applying with GCS (Glasgow Coma Scale) score. Twenty patients with severe TBI whose the GCS score ≤ 8 between June 2015 and June 2016 were enrolled in RD Kandou Hospital, Manado, Indonesia. Patients were randomized into two groups, with and without the mild hypothermia therapy (34-36°C) which was investigated within 24 and 72 h. The MMP-9 level was estimated using enzyme-linked immunosorbent assay (ELISA). The assessments of outcomes were determined using the GCS score within 24 and 72 h during the mild hypothermia therapy. Our results showed that the level of serum MMP-9 was decreased significantly within 72 h in the mild hypothermia therapy group. The effects of the mild hypothermia therapy toward to the GCS score alteration were noticeable increased and differences were significant in the two groups within 72 h. We concluded that the mild hypothermia therapy diminished MMP-9 protein level and improvement of the GCS score in severe TBI patients within 72 h.

Keywords: *mild hypothermia, MMP-9, GCS, TBI*

Cite This Article: Eko Prasetyo, Andi Asadul Islam, Mochammad Hatta, Djoko Widodo, and Ilhamjaya Pattelongi, "Downregulation of MMP-9 Level and GCS Score Improvement in Severe Traumatic Brain Injury Due to the Mild Hypothermia Therapy." *American Journal of Medical and Biological Research*, vol. 5, no. 2 (2017): 18-22. doi: 10.12691/ajmbr-5-2-2.

1. Introduction

The secondary insult (secondary damage, delayed non-mechanical damage) represents continuous pathological processes initiated at the moment of injury with the delayed clinical presentation. Cerebral ischemia and intracranial hypertension refer to secondary insults and, in treatment terms, these types of injury are sensitive to therapeutic intervention [1].

In recent experimental studies have suggested the participation of MMP in TBI [2,3,4]. Increased the expression and activity of MMP-9 after TBI in animals has been shown to contribute to blood-brain barrier degradation, cerebral edema, neuronal death and the associated severity of neurological deficits [5]. However, the mechanisms underlying BBB disruption are influenced by many factors, numerous studies have focused on the role of MMP-9 as a major contributor to BBB disruption. MMP-9 can degrade crucial components of the cerebrovascular matrix including collagen, laminin, and tight junction proteins such as zonula occludens-1 (ZO-1), leading to disruption of BBB (blood-brain barrier) and exacerbation of edema in acute brain injury [6].

After TBI, MMP-9 become dysregulated which up-regulation activities MMP-9 due to the activity of transcriptions factor, activator protein-1 (AP-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). The activity of AP-1 and NFκB as regulators will affect in signal pathway mitogen activated protein kinase (MAPK) particular regulation by extracellular signal-regulated kinase (ERK1/2) MAPK in MMP-9 transcriptional level [7,8,9,10,11].

The mild hypothermia therapy could be a useful method for improving the outcome of neurological treatment in patients with severe TBI [12,13,14,15,16]. Several mechanisms thought to underlie this effect which includes the reduction in the metabolic rate, attenuation excitatory amino acids and free radicals synthesis, suppression inflammatory responses, and prevention of blood-brain barrier disruption and brain edema [17,18,19,20], following ischemia-reperfusion by decreasing of the MMP-9 expression [21,22,23,24].

The GCS score, since its introduction in 1974, has frequently been used as one of the most significant

predictors of outcome after TBI [25,26]. The GCS remains a key measure in neurological assessment after TBI, and in most studies classification of the severity of the trauma is still based on the admission GCS [26].

We investigated the hypothesis that the mild hypothermia therapy could decline level of MMP-9 protein and improve neurological outcome determined by using the GCS score. The purpose of this study that the level of MMP-9 and the GCS score can be utilized as a predictor value of outcome in severe TBI patients during the mild hypothermia therapy.

2. Materials and Methods

2.1. Collection of Samples

This study was carried out base on prospective analysis of 20 patients admitted to our hospital from June 2015 to June 2016. It was designed as a randomized controlled trial to assess the effect of the mild hypothermia therapy toward biomarker in severe TBI patients.

A population with severe closed TBI (the GCS score ≤ 8) admitted within 2 hours of trauma were investigated. The predetermined entry criteria, in addition to closed TBI, they were age 16 to 45 years and no the other chronic illness. Patients who had life threatening injuries to other organs or systolic blood pressure less than 90 mmHg after resuscitation and planned surgical decompression were excluded. Those for whom randomly enrolled in the mild hypothermia therapy (n=10) and control group (n=10). Investigation of the level of MMP-9 protein prospectively and neurologic outcome determines using to the GCS score. The peripheral venous blood samples of two groups were simultaneously achieved within 24 and 72 h during the mild hypothermia therapy.

Treatment in neurosurgical intensive care unit was directed by a protocol based on the guidelines for management of severe TBI followed the patients before applying the mild hypothermia therapy and which was maintained unchanged throughout the study period. The mild hypothermia therapy was induced by surface cooling, which was initiated within 2 h after injury. The mild hypothermia (34°-36°C) procedure accomplished by placing a cooling blanket and ice pack were used to reduce the whole body temperatures. The core temperature monitored by a rectal thermometer probe was set 34°-36°C which achieved within 2 hours and the target temperature was maintained within 72 h. After finishing phase of the mild hypothermia therapy, the patients were gradually rewarmed at a rate of 1°C every 6 hours. The quality outcome of each patient was assessed by a study doctor within 24 and 72 h according to the GCS score.

2.2. Laboratory Procedure

In two groups, samples peripheral venous blood for MMP-9 were drawn between 24 and 72 h. Serum samples were collected under sterile conditions. Serum was separated immediately by centrifugation at 3000 rpm for 10 minutes and stored at - 80°C until the analysis was completed. The level of serum MMP-9 protein was measured using a calibrated instrument, Human MMP-9 Quantikine ELISA kit (catalog no. DMP 900) R & D Systems Inc., MN, USA. Procedure inspection following the procedures by manufacturer's instructions read using ELISA Reader 270 (Biomerieux, France).

2.3. Statistical Analysis

All values are presented as a mean ± standard deviation. Data entry and analysis was done using SPSS software V.20.0 (SPSS Inc., Chicago, IL., USA). Student's t-test for unpaired results. Categorical data were analyzed by Fisher's exact test. Time course differences in the parametric were compared by a nonparametric Wilcoxon rank-sum test. The results of the two groups were compared by using the Mann-Whitney U one-way analysis. A P value less than 0.05 was considered significant.

2.4. Ethical Clearance

The study was approved by ethical committee of Kandou General Hospital Human Research Review Committee. Written informed consent was obtained from their family members for inclusion in the study.

3. Results

3.1. Characteristics of Subjects

The patient's characteristic showed in Table 1. There was no significant difference in the characteristics between the two groups (p> 0.05). The two groups can be considered as homogeneous groups based on the characteristics of sex, age, and onset time of hospitalization.

Table 1. Baseline characteristics of patient in subgroups

Variables	Group		p
	Controls (n = 10)	Hypothermia (n = 10)	
Gender; (M / F)	6/4	7/3	0.500 *
Age (years) Mean (SD) / Min-Max	29.1 (8.5) / 20-44	29.3 (8.4) / 20-43	0.958 **
Onset (min): Mean (SD) / Min-Max	75.0 (21.2) / 45-120	79.0 (22.8) / 45-120	0.690 **

*Fisher's Exact **Independent t test.

3.2. The Effect of the Mild Hypothermia Therapy in the Level of MMP-9 Protein in Severe TBI Patients

There was no significant difference in the level of MMP-9 protein between the mild hypothermia therapy and control groups within 24 h (p> 0.05) as presented at Table 2 and Figure 1. The significant different level of MMP-9 protein has occurred within 72 h (p<0.05). The level of MMP-9 protein in the mild hypothermia therapy group (309.98 ± 226.84) pg/mL are lower than control (553.37 ± 198.87) pg/m within 72 h. The level of MMP-9 protein was not significantly decreased (p >0.05) with mean level of -150.59 pg/mL within 24-72 h and meanwhile in the control group, the level of MMP-9 protein was upregulated significantly (p <0.05) with mean level of 98.10 pg/mL our data explained that the mild hypothermia therapy downregulated the level of MMP-9 protein significantly within 72 h.

3.3. The Effect of the Mild Hypothermia Therapy to the GCS Score in Severe TBI Patients

The effect of the mild hypothermia therapy to the GCS score can be seen in the resume of the analysis in Table 3 and Figure 2 in which according to the GCS score difference between the mild hypothermia therapy and control group of observation within 24-72 h.

Summary results of the analysis in Table 3 and Figure 2 defined that in the mild hypothermia therapy and control groups shown have not significant effects of the change in the GCS score (p> 0.05) within 24 h. The different effect of the mild hypothermia therapy and control groups to the GCS score was significantly increased (p <0.05) within 72 h. During the period of 24 -72 h, the mild hypothermia therapy group, there was significantly raised almost 3 fold of the GCS score (p <0.05) by mean of 6.2 than the control by mean of 3.0.

Table 2. The changes level of MMP-9 protein in subgroups

Group	(Mean±SD) MMP-9 (pg/mL)			p
	24 h	72 h	Δ	
Control	455.27 ± 74.76 [b]	553.37 ± 198.87 [a]	98.10 [q]	0.037**
Hypothermia	460.57 ± 62.00 [b]	309.98 ± 226.84 [c]	-150.59 [p]	0.203**

The same *superscript* in the same column shows the results of *Independent t test* or the *Mann Whitney U test* was not significant (p> 0.05); whereas it's different, the means significant difference (p <0.05).
** *Wilcoxon test.*

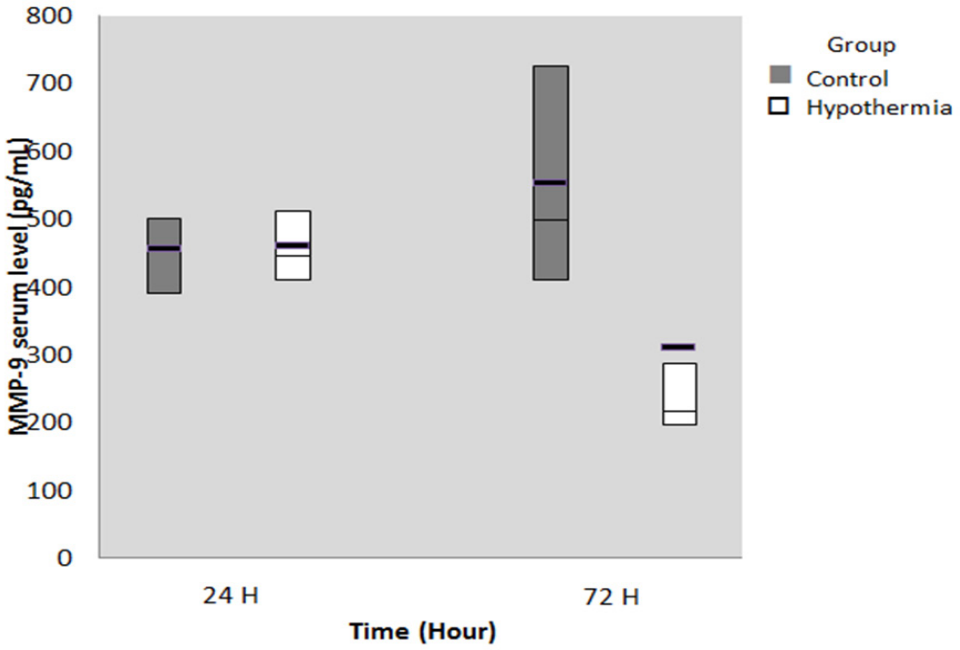

Figure 1. The changes level of MMP-9 protein serum in the two groups within 24-72 h

Table 3. The comparison of the GCS changes in subgroups

Group	(Mean ± SD) GCS			p
	24 h	72 h	Δ	
Control	5.7 ± 0.48	8.7 ± 1.77	3.0	0.005 **
Hypothermia	5.7 ± 0.48	11.9 ± 2.77	6.2	0.005 **

The same *superscript* in the same column shows the test results *of independent t test* or the *Mann Whitney U test* was not significant (p> 0.05); whereas it's different, the means significant difference (p <0.05). ** *Wilcoxon test.*

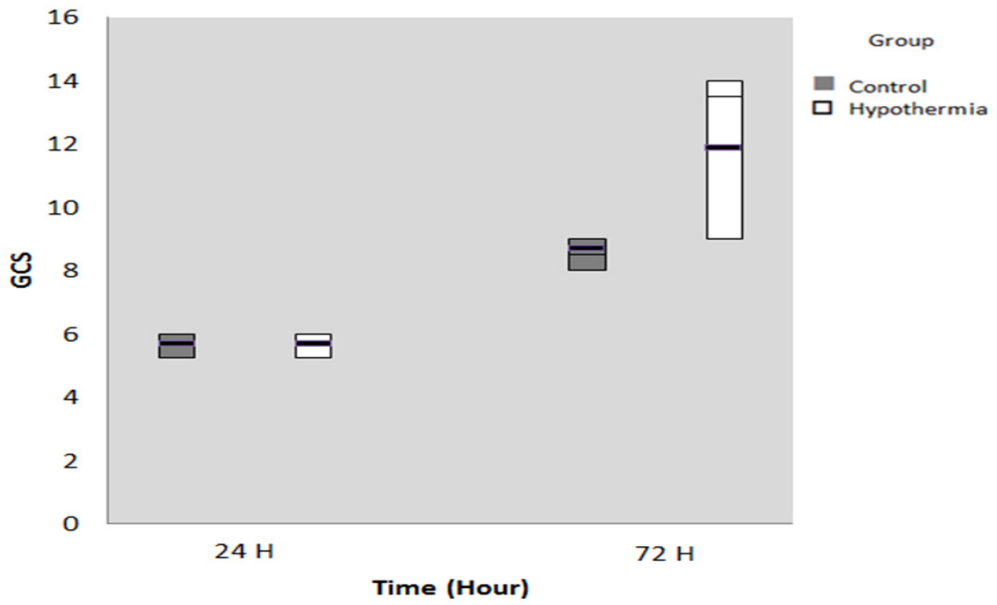

Figure 2. The changes of the GCS score in both groups within 24-72 h

4. Discussion

We have analyzed 20 patients to investigate the effects of mild hypothermia therapy in the level of MMP-9 protein and to the GCS score in severe TBI patients. We expect that it can be used as a predictor value of outcome in severe TBI patients. Our study found that mild hypothermia therapy significant downregulated the level of MMP-9 protein, and raised off the GCS score within 72 h.

In our study performed that the different level of MMP-9 protein in two groups were not significantly ($p>0.05$) within 24 h, which was distinct from the occurs in the period of 72 h ($p< 0.05$). At the period of 24 to 72 h MMP-9 protein level in mild hypothermia group decreased but not significantly ($p>0.05$). The different level of MMP-9 protein in two groups was significant within 72 h.

The downregulated of the level of MMP-9 protein due to the mild hypothermia therapy within 72 h. It was revealed that TBI induced significant increases in MMP-9 protein level in brain tissue within 24-72 h due to secondary damage of TBI [27]. The evolution support an important point neuroprotective treatments for TBI should be extended up to 72 h [1]. In theory protective effects could be achieved for a period up to 48-72 h as this is the period during which secondary brain injury develop [29]. It was the basic concepts, most studies used cooling within 24-48 h range, while some studies have used a cooling period longer than 48 h [17,18]. One reason for using more long-term cooling is that cerebral swelling and edema often are greatest 3-5 days after injury [30]. Peterson et al. reported that reduction in risk mortality was highest and favorable neurologic outcomes much more widespread when hypothermia was maintained for than 48 h. We found no evidence of complications related to the mild hypothermia therapy [31].

The ERK and MAPK signaling pathways are rapidly upregulated after TBI [9]. The several studies have reported that TBI induces ERK phosphorylation in the cortex after cortical impact injury. Activation ERK-MAPK signaling pathway significantly influenced by hypothermia and rewarming [10]. Afterward, inhibition of ERK-MAPK signaling pathway reduced the trauma-induced level of MMP, suggesting that pathway trigger upregulates of MMP-9 after TBI. The ERK1/2 specific phosphatase enzyme and the balance activity of kinase to phosphatase of ERK-MAPK signaling pathway during hypothermia therapy were temperature sensitive [32]. The hypothermia and rewarming boost this induction of inflammatory enzyme activation [10].

In this regard, the mild hypothermia therapy was decreased the expression MMP-9 that it inhibits the inflammatory response by preventing activation of the transcription factor, NFκB. The transcription control of MMPs is mediated by AP-1, NFκB. The mild hypothermia therapy has recently been reported to reduce the activation of NFκB after cerebral ischemia [33,34]. NFκB is known as a regulator of the MMP-9 promoter [35,36]. Moreover, NFκB could also promote neuronal cell death in vitro and numerous target gene of NFκB are upregulated in the acute phase and involved in inflammatory responses that potentiate ischemic injury in cerebral ischemia. Eventually, the dynamic interaction between transcription factors and signal transduction pathways are fundamental role of the mild hypothermia therapy [32,33,37].

Our current study has also been reported that the effect of the mild hypothermia therapy in severe TBI patients was appraised using the GCS score. The GCS remains a key measure in neurological assessment after TBI. Balistreri et al. found a significant correlation between the GCS and GOS for the first five years (overall 1992-1996) and consistent lack of associations from 1997–2001 [25]. Udekwu et al. reported that the average functional outcome indicates a substantial likelihood of good outcomes in survivors with low GCS scores, further complicating the use of the GCS score in the prediction of poor outcome at the time of initial patient evaluation [26].

Based on the research results, we verified that the mild hypothermia therapy was induced the GCS increased significantly ($p<0.05$) in the severe TBI patients within 72 h.

5. Conclusion

This study demonstrated that the mild hypothermia therapy diminished MMP-9 protein level and improvement of the GCS in severe TBI patients within 72 h. This study suggested that the mild hypothermia could be useful improving the level of consciousness patients with severe TBI.

Conflict of Interest

All authors have no conflicts of interest to declare.

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