



The Effect of Minocycline on MMP-9 Levels in Traumatic Brain Injury: An Experimental Study in Wistar Rats

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Abstract

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BACKGROUND: Cerebral edema in traumatic brain injury (TBI) is caused by hyperpermeability of the bloodbrain barrier (BBB). One of the causes of impaired BBB permeability is the activation of matrix metalloproteinase 9 (MMP-9), which can be inhibited by the antibiotic minocycline.

AIM: This study aimed to investigate MMP-9 levels and the effect of minocycline administration in a Wistar rat model of traumatic brain injury.

METHODS: A modified Marmarou model of brain injury in rats was used in this study. The post-test control group design consisted of an untreated control group (Group 1), a group with traumatic brain injury (Group 2), and an injury group that received minocycline (Group 3). Sandwich ELISA was used to evaluate MMP-9 levels.

RESULTS: The mean level of MMP-9 in Group 2 was 0.610 ng/ml, compared with 0.519 ng/ml in the controls, and 0.552 ng/ml in Group 3. Statistical analysis showed a statistically significant relationship between the administration of minocycline and the decrease in MMP-9 levels in Wistar rats with traumatic brain injury ($p = 0.001$).

CONCLUSION: Administration of minocycline to Wistar rats with traumatic brain injury resulted in significantly lower levels of MMP-9. This result showed that minocycline has the potential to inhibit increased MMP-9 levels in traumatic brain injury.

Introduction

Traumatic brain injury is a complex disorder caused by external forces that cause changes in brain function or brain pathology [1]. As many as, 1.7 million people annually experience traumatic brain injury in the United States [2], [3]. Meanwhile in Indonesia, the prevalence of traumatic brain injury increased from 14.5% in 2007 to 14.9% in 2013 [4].

Recent studies have shown that overexpression of molecular matrix metalloproteinase-9 (MMP-9) occurs as a result of cerebral edema and damage to the blood-brain barrier (BBB) in traumatic brain injury [5], [6]. Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes that play a role in the degradation and remodeling of the extracellular matrix in both physiological and pathological processes. A major function of MMPs is degradation of the extracellular matrix to facilitate cell migration and invasion. However, uncontrolled expression of MMPs can result in tissue

injury and destruction. As MMP regulation is complex and tightly regulated, loss of this regulatory control is likely to play a role in the pathophysiology of BBB damage in cerebral edema. Therefore, MMP-9 inhibition is an important target in the treatment of traumatic brain injury [5].

Of the tetracycline derivatives capable of inhibiting MMP-2 and MMP-9, minocycline is the strongest non-specific MMP inhibitor, reducing invasion and migration in cancer cells and exerting anti-inflammatory effects in normal tissue [7], [8]. Therefore, researchers are interested in understanding the effects of minocycline administration on MMPs in the context of cerebral edema following traumatic brain injury. Until recently, there have been few studies on MMP-9 inhibitors. In addition, well-known MMP-9 inhibitors are not widely available and are unaffordable in developing countries. This study aimed to determine the impact of traumatic brain injury on MMP-9 levels as well as the effects of minocycline administration on MMP-9 in a Wistar rat model of traumatic brain injury.

Materials and Methods

This research was conducted on rats using an experimental laboratory method with a post-test control group design. The research was conducted at the Animal Laboratory, Faculty of Medicine, Hasanuddin University over a period of 2 weeks in September 2020. The research population consisted of rats with traumatic brain injury and a control group of uninjured rats. The sample size for each group was determined using Federer's formula [9], [10], [11], which identified the minimum number of experimental animals per group as nine (a total of 27 rats for this study). Inclusion criteria in this study were male Wistar rats (*Rattus norvegicus*) aged approximately 3–4 months, body weight approximately 300–400 g, and good health status. Rats that looked sick during the adaptation period (inactive) and showed weight loss > 10% during adaptation were excluded from the study. Experimental animals fulfilled the study dropout criteria if they died before the study was completed or the blood sample was damaged. Animals that met the inclusion criteria were included in the traumatic head injury model (craniectomy and traumatic brain injury). The rats were randomly assigned to three groups: Group 1 (control) = no head trauma applied with blood samples taken at the same time as Groups 2 and 3; Group 2 (TBI) = rats undergoing craniectomy and direct trauma to the brain with blood samples taken 24 h after treatment; and Group 3 (TBI+M) = craniectomy and direct trauma to the brain followed by administration of minocycline (10 mg/kg) and collection of blood samples 24 h after treatment. The blood samples were collected from the tail vein.

Minocycline was purchased from Dexa-Medica Pte.ltd. Levels of MMP-9 were determined using an ELISA kit (E0321Ra) from Bioassay Technology Laboratory (Shanghai Korain Biotech Co., Ltd, Shanghai, China).

Surgical procedure

The rats were anesthetized with 3% of sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. A midline incision was made and the skin and temporal muscles were reflected before opening the skull. Craniectomy was performed with a diameter of 4.0 mm, using a dental bur on the left side. Briefly, a modified Marmarou weight drop model [12], [13] was used to apply load to the brain (tip diameter, 4.0 mm; cortical contusion depth, 5.0 mm; impact velocity, 6.0 m/s; residence time, 50.0 ms). After the injury, the skin incision was closed with nylon sutures 5/0. Minocycline [13], [14], [15] was administered as a single oral dose (10 mg/kg) to Group 3 (TBI+M) animals 12 h after the procedure.

Sample processing

The blood was centrifuged and the resulting supernatant (designated plasma) was immediately transferred into a clean polypropylene tube using a Pasteur pipette. The samples were then maintained at 2–8°C. Levels of MMP-9 were measured using the sandwich ELISA method. The levels of MMP-9 from each sample were compared between groups.

Statistical analysis

All data were processed and analyzed using SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Initially, data distribution was analyzed using the normality test (Shapiro–Wilk) for parametric data and the homogeneity test (Levene) for non-parametric data with $p > 0.05$. As the data were normally distributed, statistical analysis was continued to compare the mean differences between variables. This analysis used one-way analysis of variance (ANOVA) and the Fisher's least significant difference (LSD) *post hoc* test for parametric data.

The research was carried out after ethical clearance from the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University Makassar, under number 786/UN4.6.4.5.31/PP36/2020.

Results

Characteristics of the animal models used, such as body weight, are presented in Table 1. The Lavene's homogeneity test was used to determine the homogeneity of Wistar rats used as brain injury models, and $p > 0.05$ was found, indicating that there was no significant variation between each body weight.

Table 1: Wistar rats body weight

Body weight (g), mean \pm SD	p
390.08 \pm 10.58	0.155

SD: Standard deviation.

The experimental animals were divided into three groups, with nine rats per group. As the data were normally distributed, ANOVA was used for statistical analysis. The mean MMP-9 level in the injury group (TBI) was 0.610 ng/ml, while the mean value in the uninjured controls was 0.519 ng/ml (Table 2). In Group 3 (TBI+M), the MMP-9 level was 0.552 ng/ml. The differences were

Table 2: Comparison of matrix metalloproteinase 9 level between groups

Group	MMP-9 levels (ng/ml)				p
	n	Mean \pm SD	Minimum	Maximum	
1 (control)	9	0.519 \pm 0.024	0.483	0.562	0.001
2 (TBI)	9	0.610 \pm 0.068	0.492	0.671	
3 (TBI+M)	9	0.552 \pm 0.039	0.468	0.605	

TBI: Traumatic brain injury, MMP-9: Matrix metalloproteinase, 9SD: Standard deviation.

significant, as shown by ANOVA ($p = 0.001$). Therefore, there was a statistically significant relationship between the administration of minocycline and the observed decrease in MMP-9 levels in cerebral edema in Wistar rats.

Table 3 shows the results of *post hoc* analysis using the LSD test. Analysis of MMP-9 levels in Group 2 (TBI) and the control group showed that the difference was statistically significant ($p < 0.001$). Meanwhile, the levels of MMP-9 in Group 2 (TBI) and Group 3 (TBI+M) were also significantly different ($p = 0.033$).

Table 3: Post hoc comparison using least significant difference

Groups	$p < 0.05$
Group 1 (control)	
Group 3 (TBI+M)	0.029
Group 2 (TBI)	< 0.001
Group 3 (TBI+M)	
Group 2 (TBI)	0.033

TBI: Traumatic brain injury.

Discussion

In this study, we showed that traumatic brain injury in Wistar rats resulted in increased serum levels of MMP-9. Administration of minocycline following injury led to a significant reduction in MMP-9. The injury was applied directly to the brain rather than the head to enable observation of the real impact of direct brain trauma on MMP-9 levels. Trauma given to the head can result in more diverse manifestations such as epidural hemorrhage, subdural hemorrhage, subgaleal hemorrhage, and skull fracture.

Marmarou's drop model (weight drop traumatic brain injury model) was used to induce the effects of BBB damage caused by traumatic brain injury in Wistar rats [11], [16]. Levels of MMP-9 were significantly increased in Group 2 rats 24 h after traumatic brain injury (impacted with a force of 571.17 Newton/mm² in the craniectomy area), compared with levels in the control group. This is in line with the study of Hayashi *et al.* in 2009, which showed that MMP-9 levels were significantly upregulated 24 and 48 h after traumatic brain injury [17].

Overexpression of MMP-9 is induced by excitotoxicity, neuroinflammation, oxidative stress, and apoptosis as a result of ischemic reperfusion in secondary injury. Secondary injury occurs within minutes of traumatic brain injury and can last for months to years, playing a major role in the morbidity and mortality resulting from traumatic brain injury [18]. It is important to recognize the point at which serum levels of MMP start to become unstable after traumatic brain injury, as this is the optimal time to administer MMP inhibitors [17].

Increased levels of MMP cause hyperpermeability of the BBB, leading to cerebral edema [19]. Therefore, suppression of MMP expression is

the key to endogenous neuroprotection. MMP regulation can occur at multiple levels, including gene expression, post-translational processing, compartmentalization, and regulation by endogenous tissue inhibitors of matrix metalloproteinases [20]. Longstanding treatment techniques for traumatic brain injury associated with MMP inhibition include hyperbaric oxygen therapy and hypothermia [17]. In addition, traditional medicines such as rhubarb (dried roots and rhizomes) can reduce levels of MMP-9 [21]. Administration of tetracycline antibiotics such as minocycline and doxycycline can also reduce MMP-9 levels in traumatic brain injury [22].

In this study, minocycline was administered orally at 10 mg/kilogram body weight following brain injury to determine its effect on MMP-9 levels. *Post hoc* analysis revealed that the effect of minocycline on MMP-9 levels in the injured rats was significant. Thus, oral administration of minocycline reduced MMP-9 levels in this model of traumatic brain injury.

Minocycline is a member of the tetracycline group of antibiotics, which inhibit MMPs by affecting transcription and activation [23]. This is likely due to their ability to bind to zinc, which is required for the activation of MMPs [22]. Minocycline is the strongest non-specific tetracycline MMP inhibitor capable of inhibiting MMP-9 [7], [8]. Minocycline is highly lipophilic, meaning it can easily cross the BBB. Maximum concentration is observed within 2–3 h of ingestion, while its half-life (12–18 h) is longer than that of first-generation tetracyclines [24], [25], [26].

The limitation of our study was that it did not compare the effect of minocycline with standard MMP-9 inhibitors due to the lack of availability of such inhibitors in our institution. Therefore, further studies have been planned.

Conclusion

The levels of MMP-9 in an animal model of brain injury were significantly reduced by minocycline treatment. This result showed that minocycline has the potential to inhibit the MMP-9 increase that occurs in traumatic brain injury. We suggest that further research is needed to identify other factors that may influence MMP-9 levels in this context. In addition, research into the effects of minocycline on MMPs in human traumatic brain injury is required.

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