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The Analysis of the Effective Systemic Lidocaine Dosage on the Expression of HMGB1 mRNA on Mice with Sterile Musculoskeletal Injury

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Abstract

A severe injury can trigger an inflammation response and excessive response can cause multiple organ failure. HMGB1 is an early inflammation mediator in sterile injury and a late inflammation mediator in infection. It is an impor- tant mediator in severe sepsis and always associated with the severity of organ failure. Previous studies showed that the administration of systemic lidocaine could inhibit the expression of HMGB1 on septic mice with musculoskeletal injury. Nine male adult Balb/c mice were grouped by simple random sampling method into three groups of intravenous lidocaine injection dosages: 2 mg/kg, 3 mg/kg, 4 mg/kg. Musculoskeletal injury was done by breaking the left femo- ral bone in a close manner. Peripheral blood sampling was done 4 hours after injury and 2 hours after lidocaine therapy. To evaluate the expression level of HMGB1 mRNA, RT-PCR was used. The result of our study showed that intra- venous lidoaine administration on the 3 groups could decrease the level of HMGB1. In conclusion, lidocaine hold an important role in clinical diseases by inhibiting HMGB1.

Keywords

High Mobility Group Box 1, Lidocaine, Musculoskeletal Injury

1. Introduction

A severe injury can trigger an inflammation response and excessive response can

produce multiple inflammation cytokines that can activate the systemic response in term of systemic inflammation response syndrome (SIRS). SIRS can affect the vascular permeability, and the function of heart, lungs, liver, kidneys, intestines, and other organs; and induce metabolic changes that can cause multiple organ failure (MOF), septic shock, and even death [1] [2] [3].

Previous study showed that inflammation cytokine like high mobility group box 1 (HMGB1) was always associated with organ worsening [2] [4]. HMGB1 is a nuclear protein that has been known as transcription and growth factor [5] [6] [7]. HMGB1 is an important inflammation mediator in sepsis and the level is increased in major surgery procedures [8] [9] [10].

Lidocaine is a local anaesthetic from amide group that has been long known and used in clinical practice to prevent surgical pain and reduce the pain from disease proccess. Lidocaine also has anti-arrythmic and anti-inflammation properties. In the last decades, the anti-inflammation property of lidocaine had been used to treat septic mice and protect against organ failure from its ability to inhibit the expression of macrophage HMGB1 [11]-[16].

The purpose of this study is to analyze the effectivity of systemic lidocaine dosages of 2 mg/kg body weight, 3 mg/kg body weight, and 4 mg/kg body weight on the expression of mice Balb/c mice with closed femoral fracture.

2. Material and Methods

This is a longitudinal laboratory experiment on animals, using nine Balb/c mice. The inclusion criteria including: healthy male Balb/c mice, body weight 35 - 40 grams, aged 7 - 8 weeks. Healthy Balb/c mice are mice with shiny eyes, undull fur, active movement, and good appetite. Dead mice on this study process will be excluded. Mice were obtained from the maintenance and development unit of experimental animal laboratory in Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. These experimental animals were adapted and carried out according to the Declaration of Helsinki.

The sample size was determined simply according to the Ethical Committee of Animal Experiment in Hasanuddin University Faculty of Medicine, Makassar, Indonesia. Every group consisted of 3 mice. The study was conducted in Molecular Biology and Immunology laboratory in Hasanuddin University Faculty of Medicine Makassar on August 2015. The lidocaine used was 2% isobaric lidocaine, produced by Kimia Farma Indonesia. The intervention manner was adjusted with the mice taking manner from its cages.

Firstly, 0.3 ml venous blood was taken from every mice' tails to evaluate the initial expression of HMGB1 mRNA (Group I), then the mice were anaesthesized by injecting 50 mgs/kg body weight of Ketamine intraperitoneally. After that, mice left femoral bone were broken in a close manner using 2 Kocher, and after 4 hours 0.3 cc blood was taken (Group II). Mice were injected with systemic lidocaine every 2 hours for 24 hours from the tail veins with dosages of 2 mgs/kg body weight, 3 mgs /kg body weight, and 4 mgs/kg body weight. Two hours after lidocaine therapy, 0.3 cc mice blood were drawn again for the third

examination (Group III). All blood samples were mixed with L6 solution to be processed into nucleic acid extract and stored −80˚C temperature.

2.1. Real-Time Polymerase Chain Reaction (RT-PCR) Analysis

RT-PCR analysis was done by making specific primers PCR mixture as much as 22.5 µl. And then 1 µM concentrations of the forward and reverse specific primers of mice HMGB1 gene were added (HMGB1 for, 5'-CGTCTGGCTCCCGCTCT-CACA-3' and Rev: 5'-GAGTCGCCCAGTGCCCGTC-3'. Beta actine: for: 5'-CTG-AGAGGGAAATCGTGCGT-3' and Rev: 5'-CCACAGGATTCCATAC CCAAGA-3 as housekeeping gene). After that, about 2.5 µl DNA extract was added into the 22.5 µl specific PCR mixture and first stage amplification was done with 94˚C for 2 minutes and continued by 40 cycles of 60 seconds in 94˚C, 45 seconds in 57˚C, using Real time PCR machine (Bio-Rad CFX Connect, USA). The PCR result was detected using SYBR Green and analyzed using Bio-Rad CFX Manager 3.1 software Statistical Analysis. Normal distribution data was expressed in mean ±SD. The data was analyzed using ANOVA test or its alternative with significance level of $p < 0.05$.

3. Results

The mean weight of the 9 mice from the 3 experimental mice was: control group 36.2 grams, intervention group 37.466 grams, and therapeutic group 38.8 grams. There was no significant difference in the mice weight in the 3 experimental groups ($p < 0.05$).

The expression of HMGB1 mRNA had been detected by RT-PCR examination before the breaking of left femoral bone. The expression of HMGB1 mRNA level in Group I: early 7.05 ± 0.081; intervention 12.653 ± 0.477; after lidocaine therapy 7.185 ± 0.045. In Group II, the expression of HMGB1 mRNA was: early 6.968 ± 0.071; intervention 12.504 ± 0.111; after lidocaine therapy 6.402 ± 0.100. In Group III, the expression of HMGB1 mRNA: early 6.981 ± 0.105; intervention 12.501 ± 0.066; after lidocaine therapy 6.164 ± 0.129. The expression of HMGB1 mRNA level was markedly increased in 4 hours after left femoral bone fracture. The high level of HMGB1 mRNA was significantly decreased for 24 hours after systemic lidocaine therapy ($p < 0.05$). The result of HMGB1 mRNA expression profile in mice blood can be seen in Table 1.

Figure 1 revealed that the intravenous lidocaine administration by doses 2 mg/kg, 3 mg/kg, 4 mg/kg on the 3 groups significant decrease of the level HMGB1.

4. Discussion

Inflammation response is a very complex series of events involving many inflammation, anti-inflammation process, humoral and celullar process that will cause metabolic abnormality in systemic circulation [3] [4] [17] [18] [19] [20]. Continuous and excessive inflammation response will cause multiple organ failure that can cause death [2] [5] [6] [7]. Severe injury will stimulate the body immune system to produce inflammatory cytokines, such as TNF α, IL1, and HMGB1 [20]

Table 1. Mean mice HMGB1 mRNA expression profile in each group.

Group	mRNA HMGB1 Expression		
	Control (before Injury)	Intervention (4 Hours after Injury)	Therapy (2 Hours after Lidocaine Therapy)
Group 1	7.05 ± 0.081	12.653 ± 0.477	7.185 ± 0.045
Group 2	6.968 ± 0.071	12.504 ± 0.111	6.402 ± 0.100
Group 3	6.981 ± 0.105	12.501 ± 0.066	6.164 ± 0.129

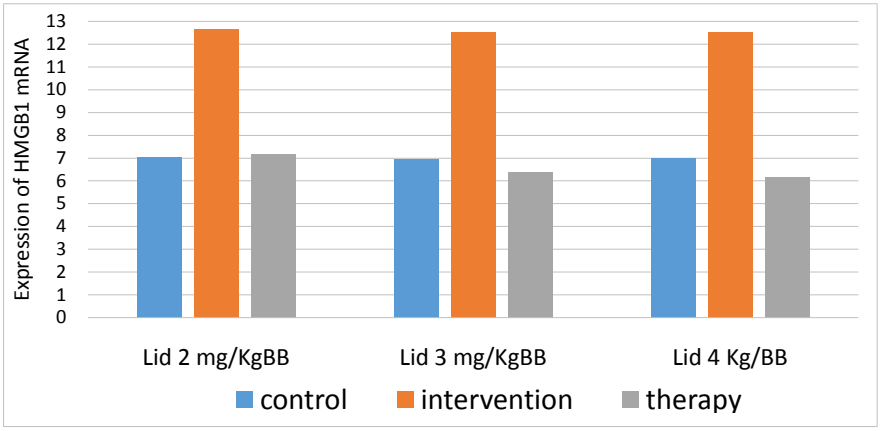

Figure 1. The expression of HMGB1 mRNA in each group (n = 9, $p < 0.05$).

[21] [22] [23]. HMGB1 is a non-histone nuclear protein that can be found in almost all mammalian cells and is known to be the early pro-inflammatory mediator in sterile injury and late pro inflammatory mediator in infection [1] [2] [3] [4].

Data of our study showed that HMGB1 mRNA expression can be detected before mice underwent close femoral fracture, and the level will be increased after the mice underwent left femoral bone fracture. Increased level of HMGB1 mRNA expression was detected 4 hours after left bone fracture. The result of this study was consistent with previous studies which showed that increased HMGB1 level can be detected on the end of surgery and can last up to 2 days after liver resection surgery or on the first day of heart bypass procedure.

Lidocaine is a traditional local anaesthetic that has been long used as analgesic and anti-arrythmia. The anti inflammatory property of lidocaine has long been known and proved useful in the treatment of chronic diseases, such as rheumatic arthritis and colitis [10] [11] [12] [13] [14].

The result of our study showed that the administration of systemic lidocaine in dosages of 2 mgs/kg body weight, 3 mgs/kg body weight, and 4 mgs/kg body weight every 2 hours for 24 hours after musculoskeletal injury can decrease the expression of HMGB1 mRNA up to the initial level before mice underwent musculoskeletal injury.

The result of lidocaine therapy in this study was consistent with previous studies on septic experimental animals which was induced with lipopolysaccharides

(LPS) or cecal ligation and puncture (CLP). The administration of lidocaine can inhibit the production of HMGB1 and protect mice against septic shock [24] [25] [26] [27] [28]. We conclude that lidocaine is useful and has an important role in the management of many clinical diseases by inhibiting the production and release of HMGB1 in pathological condition.

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