

Profile of HMGB1 mRNA Expression and TLR4 Protein in BALB/c Mice Model Sterile Injury after Systemic Lidocaine Administration

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ABSTRACT

Background: High mobility group box 1 (HMGB1) is a cytokine proinflammation which contributes to inflammation. HMGB1 physically interacts with toll like receptor 4 (TLR4) to release macrophage cytokines. The aim of this study was to demonstrate the effectiveness of systemic lidocaine administration to inhibit the expression of HMGB1 mRNA and TLR4 protein in mice BALB/c mice with sterile injury. **Material and Methods:** Twenty adult male BALB/c mice were divided into lidocaine and control groups. A sterile injury is done by closed fracturing the left thigh bone of the mice. The lidocaine group was treated with 2 mg/kgBW lidocaine through tail vein injection after 4 h of sterile injury. The control group was given distilled water therapy as a substitute for lidocaine. Mice blood is extracted from the tail vein before trauma, 4 h after trauma, and 2 h after the administration of lidocaine and distilled water is complete. The HMGB1 mRNA expression was examined by quantitative real-time polymerase chain reaction (qPCR) while the TLR4 protein level was determined with enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. **Result:** The HMGB1 mRNA expression and TLR4 protein levels in BALB/c that sustained inflammation due to a sterile injury was significantly decreased in the lidocaine group ($p < 0.00$). **Conclusion:** Administration systemic 2 mg/kgBW of lidocaine is effectively inhibits HMGB1 mRNA and TLR4 protein in mice that sustain inflammation due to a sterile injury.

Keywords: HMGB1 mRNA, TLR4, lidocaine, sterile injury.

INTRODUCTION

HMGB1 is an DNA-binding nucleotide protein whose primary function is as a factor of transcriptional regulation and growth.^{1,2} HMGB1 as a co-factor nuclear acting as an intracellular messenger molecule, is released to extracellular cells when injured or infected.^{3,4} Another role of HMGB1 is pro-inflammatory cytokines that contribute significantly in many cases of inflammation and sepsis. HMGB1 interacts with toll-like receptors (TLR4) to release macrophage cytokines.^{5,6} Lidocaine have analgesic and anti-arrhythmic effects, also have anti-inflammatory properties and cascade inflammation modulating effect on model sepsis mice induced with lipopolysaccharide.^{7,8,9} Previous studies have shown that administration of systemic lidocaine can inhibits HMGB1 mRNA expression and provides a protective effect against liver, lung and cardiac ischemia in model sepsis mice.¹⁰ The purpose of this study was to determine whether the administration of systemic lidocaine dose 2 mg/kgBW for 24 h can inhibit expression of HMGB1 mRNA and TLR4 protein level in BALB/c mice who suffered sterile injury.

MATERIAL AND METHODS

This research is animal trial prospective laboratory study using healthy male BALB/c mice (weight 35-40 grams,

age 10-12 weeks; n = 20). Healthy BALB/c mice have glowing eyes, no dull feathers, active moves, and a good appetite. Mice that died during the study were excluded. Mice were obtained from the unit maintenance and development of animal trial Molecular Microbiology Laboratory and Immunology Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. The mice were adapted and treated the following procedures and principles of the Declaration of Helsinki. BALB/c mice were divided into two groups: lidocaine group and control group, each group consist 10 mice. At first, blood from the tail vein each mouse was taken as much as 0.3 ml to examine the expression of HMGB1 mRNA and TLR4 protein in first blood, mice then in anesthetized with ketamine 50 mg/kgBW intraperitoneally. Sterile injury is done by closed fracturing the left thigh bone of the mice using two needle holders. Four hours after the mice had a sterile injury, 0.3 ml blood was taken again from tail vein for a second blood examination. The lidocaine group was given therapy with 2 mg/kgBW of lidocaine (2 % lidocaine, PT Kimia Farma, Jakarta, Indonesia) through tail vein injection, once every 2 h, continuously until 24 h. The control group was given distilled water as a replacement for lidocaine therapy. Two hours after

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the treatment of lidocaine and distilled water were completed, 0.3 ml of blood was drawn again from the two study groups for a third blood test. All blood samples were mixed with L6 solution and processed into nucleic acid extract and stored at -80°C before the qPCR and ELISA were examined.

ELISA Assay

The level of TLR4 protein in the serum and was determined with ELISA kits (Life Span Bioscience, Inc. of Seattle, North America) according to the manufacturer's instructions.

Quantitative Real-time polymerase chain reaction (qPCR) Analysis

The qPCR examination was performed using a PCR Bio-Rad BR004129USA machine. A mixture of 22.5 μl PCR Mastermix and SYBR reagent was prepared. The following forward and reverse primers (1 μl each) for HMGB1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as housekeeping gene, were used: (HMGB1 for, GAG ATC CTA AGA AGC CGA GA; HMGB1 Rev, CTT CCT CAT CCT CCT ATC; GAPDH for, GAC CAC AGT CCA TGC CAT CA; GAPDH Rev, CAT CAC BCC ACA CTT TCC). Next, 2.5 μl of DNA extract was added to the 22.5 μl mixture of primary PCR mix + primary. First stage amplification was performed at 94°C for 2 s and continued until 40 cycles 60 s at 94°C , and 45 s at 57°C . Then, the results come out in the form of data.

Statistical analysis

The data were processed using SPSS software version 20. The normally distributed data were compared with a Kolmogorov-Smirnov test and presented as mean \pm SD. Comparison of data between groups were made using a t-test. A value of $p < 0.05$ was considered significant.

Ethics statement

The study was conducted after obtaining a recommendation letter of ethical clearance from the Medical Research Ethics Committee at the Faculty of Medicine, Hasanuddin University (Makassar, Indonesia) with registration number UH16050436 dated October 28, 2016.

RESULTS

The average weight of BALB/c mice in the lidocaine group was 41.3 grams and in the control group of 39.34 grams. There was no significant difference between the two experimental groups $p < 0.05$.

i. Control group:

The initial HMGB1 mRNA expression mean \pm SD was 6.75 ± 0.36 , after 4 h of sterile injury increased to 11.29 ± 0.64 and 2 h after distilled water administration increased steadily to 13.49 ± 0.45 . The HMGB1 mRNA expression increase significantly, p value < 0.00 . Preliminary TLR4 protein content of mean \pm SD is 0.31 ± 0.18 , after 4 hours of sterile injury increased to 1.67 ± 0.26 and 2 h after distilled water administration increased to 2.65 ± 0.28 . TLR4 protein level increase significantly, p value < 0.00 .

The HMGB1 mRNA expression and TLR4 protein levels in both control groups can be seen in Figure 1.

ii. Lidocaine group:

The initial expression of the mean HMGB1 mRNA \pm SD was 6.73 ± 0.66 , after 4 h of sterile injury increased to 11.90 ± 0.62 and 2 h after lidocaine therapy decreased to 6.94 ± 0.51 . The mRNA HMGB1 expression decreases significantly, p value < 0.00 . Preliminary TLR4 protein content of mean \pm SD is 0.30 ± 0.13 , after 4 h of sterile injury increased to 1.83 ± 0.24 and 2 h after

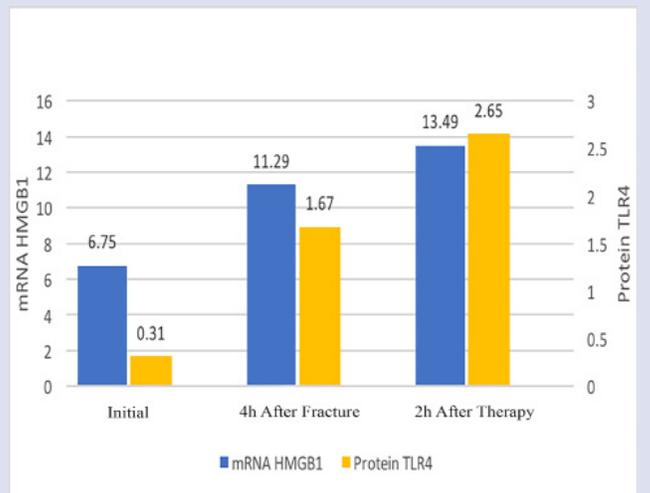


Figure 1: An expression of HMGB1 mRNA and TLR4 protein levels in both control groups

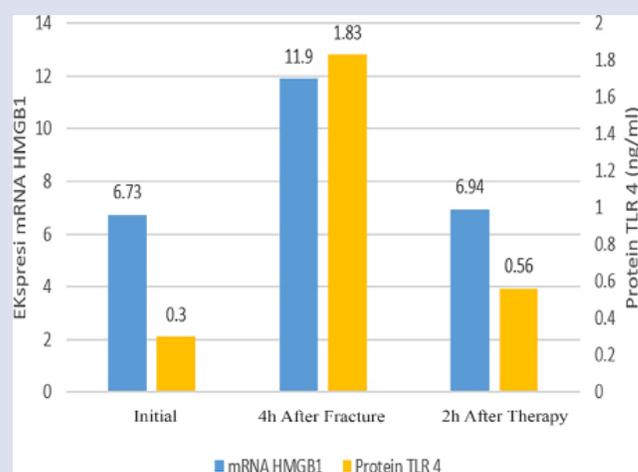


Figure 2: An expression of HMGB1 mRNA and TLR4 protein levels in both groups of lidocaine

lidocaine therapy decreased to 0.56 ± 0.17 . Protein level TLR4 decreases significantly, p value < 0.00 .

The HMGB1 mRNA expression and TLR4 protein levels in both groups of lidocaine can be seen in Figure 2.

DISCUSSION

HMGB1 is a non-histone nuclear protein abundant and can be found in almost all types of mammalian cells.¹¹ HMGB1 includes a classic pro-inflammatory cytokine mediator, HMGB1 level will increase in blood in the event of injury or infection.¹² HMGB1 is released passively from necrotic cells and actively from macrophage, monocyte, and dendritic cells.¹³ Excessive increased level of HMGB1 is always associated with worsening organ function. Extracellular HMGB1 will activate the signal path of TLR4 cell macrophages and dendrites.^{14,15}

Our results show that HMGB1 mRNA expression and TLR4 protein levels can be found in normal BALB/c mice blood. The expression of HMGB1 mRNA and TLR4 protein content increased after 4 h of BALB/c mice since sterile injury. Increase of mRNA HMGB1 expression and protein TLR4 shows that sterile injury within mice BALB/c results in severe inflammation. Control group express mRNA HMGB1 and TLR4 protein level statistically significant, $p < 0.00$. Result of this study shows that sterile aquadestila injection does not have therapeutical effect to inhibit inflammation.

The expression of mRNA HMGB1 in lidocaine group increased 1.8 times, from mean \pm SD: 6.73 ± 0.66 to 11.90 ± 0.62 , and TLR4 protein level increased 6.2 times from mean \pm SD: 0.30 ± 0.13 to 1.83 ± 0.24 . Increased expression of HMGB1 mRNA and TLR4 protein levels in the lidocaine group decreased after lidocaine therapy 2 mg/kgBW every 2 h, continuously for 24 h, each from mean \pm SD: 11.90 ± 0.62 to 6.94 ± 0.51 and from mean \pm SD: 1.83 ± 0.24 to 0.56 ± 0.17 . decreased expression of mRNA HMGB1 and protein TLR4 is significant in statistic, p value < 0.00 . Our results show that 2 mg/kgBW lidocaine administration inhibits transcription of HMGB1 mRNA expression and translation of TLR4 protein in sterile injury BALB/c mice with sterile injury.^{16,17} Previously, Hollmann MW *et al* have shown that amide groups local anesthesia inhibited release of inflammatory mediators leukotrin B4 and interleukin-1 from human neutrophils and monocytes.⁸

In a previous reasearch, Wang HL *et al* demonstrated that administration of systemic lidocaine inhibits release of HMGB1 septic mice which induced with lypopolysaccharide¹⁰ and transcription of mRNA HMGB1 in patients during hysterectomy.¹⁸ In a separate study, Liu J *et al* have shows that systemic lidocaine therapy protects dysfunction of kidney and liver in septic mice model with inhibiting expression of TLR4, NF- κ B and interleukin-6.¹⁹

While the expression of HMGB1 mRNA and TLR4 protein levels in the control group increased steadily when compared from before the injury, 4 h after injury, and 2 h after administration of distilled water, $p < 0.00$. The results of this study show that administration of distilled water does not provide a therapeutic efficacy to inhibit inflammatory process.

The results of this study are consistent with the results of previous studies showing that systemic lidocaine therapy has anti-inflammatory properties in a number of sepsis disease models in experimental animals.

Within our hypothesis that 2mg/kgBW intravenously administered lidocaine every 2 hours, continuously for 24 hours of efficacy is used to inhibit transcription of HMGB1 mRNA expression and TLR4 protein translation in sterile inflammation.

CONCLUSION

Intravenous administration 2 mg/kgBW of lidocaine therapy every 2 hours, continuously for 24 hours is effectively used to inhibits HMGB1 mRNA expression and TLR4 protein in BALB/c mice that sustain inflammation due to a sterile injury. Our results re-emphasize the importance of understanding that systemic lidocaine therapy has effect on anti-inflammatory in many conditioned diseases caused by sterile inflammation or infection.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATION USED

mRNA: messenger Ribonucleic acid, HMGB1: High mobility group box 1, TLR4: Toll like receptor 4, qPCR: quantitative Real-time polymerase chain reaction, NF- κ B: nuclear factor κ B.

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GRAPHICAL ABSTRACT

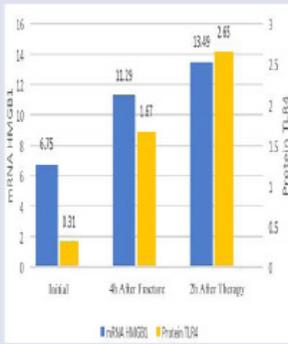


Figure 1: An expression of HMGB1 mRNA and TLR4 protein levels in both control groups

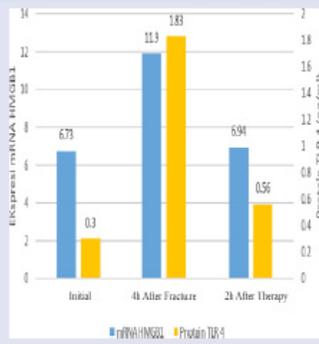


Figure 2: An expression of HMGB1 mRNA and TLR4 protein levels in both groups of lidocaine

SUMMARY

High mobility group box 1 (HMGB1) is a cytokine proinflammation which contributes to inflammation. HMGB1 physically interacts with toll like receptor 4 (TLR4) to release macrophage cytokines. Administration systemic 2 mg/kgBW of lidocaine is effectively inhibits HMGB1 mRNA and TLR4 protein in mice that sustain inflammation due to a sterile injury.

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