Original Article

Systemic lidocaine inhibits high-mobility group box 1 messenger ribonucleic acid expression and protein in BALB/c mice after closed fracture musculoskeletal injury

ABSTRACT

Background: Severe musculoskeletal trauma can trigger an inflammatory response, and an excessive inflammatory response can lead to systemic inflammatory response syndrome and multiorgan failure. High-mobility group box 1 (HMGB1) is an early mediator pro-inflammatory cytokine in sterile injuries and a late cytokine mediator in infection and sepsis. Previous research has shown that administration of systemic lidocaine can inhibit HMGB1 expression in macrophages of septic rats. The aim of this study was to demonstrate the efficacy of systemic lidocaine to inhibit HMGB1 mRNA and protein in a BALB/c mouse model of sterile inflammation due to closed fracture musculoskeletal injury.

Materials and Methods: Twenty adult male BALB/c mice were divided into lidocaine and control groups. The closed fracture musculoskeletal injury was performed by breaking the left thigh bone of the mice. Four hours after undergoing the closed fracture, the lidocaine group was treated with lidocaine intravenous (2 mg/kg). The same volume of distilled water was injected into the control group instead of lidocaine. HMGB1 mRNA expression was examined with real-time polymerase chain reaction, and HMGB1 protein level was determined with enzyme-linked immunosorbent assay.

Results: The expression of HMGB1 mRNA and protein levels in mice that sustained inflammation due to a closed fracture musculoskeletal injury was significantly decreased in the lidocaine group ($P < 0.00$ and $P < 0.00$ for mRNA and protein, respectively).

Conclusions: Intravenous administration of lidocaine effectively inhibited the inflammatory process in BALB/c mice that underwent closed fracture musculoskeletal injury by suppressing HMGB1 mRNA transcription and HMGB1 protein translation.

Key words: Closed fracture; lidocaine; mRNA high-mobility group box 1

Introduction

Severe trauma or infection stimulate the body's immune system to produce inflammatory mediators such as tumor necrosis factor alpha, interleukin-6, and high mobility group box 1 (HMGB1).[1-4] Previous studies have shown that HMGB1 increases during major surgical procedures, infectious diseases, and autoimmune diseases and will aggravate

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Robert Hotman Sirait[1,2], Mochammad Hatta[2], Muhammad Ramli[3], Andi Asadul Islam[4], Syafrie Kamsul Arief[3]

[1]Department of Anesthesiology, Faculty of Medicine, Christian University of Indonesia, Jakarta, [2]Department of Microbiology, Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Departments of [3]Anesthesiology and [4]Neurosurgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

Address for correspondence: Prof. Mochammad Hatta, Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. E-mail: hattaram@yahoo.com

multiorgan failure.[5-11] Lidocaine, in addition to its analgesic and anti-arrhythmic effects, has anti-inflammatory properties and a protective effect for acute ischemic injuries of liver, lung, and heart in a septic mouse model by inhibiting expression of HMGB1.[12-14] The present study was designed to determine the anti-inflammatory effect of systemic lidocaine in BALB/c mice with closed fracture musculoskeletal injury.

Materials and Methods

This was a prospective laboratory experimental animal study using healthy male BALB/c mice (weight 35–40 g and age 10–12 weeks). Healthy BALB/c mice have glowing eyes, no fainted fur, are active, and have a good appetite. Mice that died during the study were excluded from the study. Mice were obtained from the Maintenance and Development Unit of the Experimental Animal Laboratory of Molecular Microbiology and Immunology Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. The experiments were carried out according to the procedures and principles of the Committee for the Purpose of Control and Supervision of Experiments on Animals. The number of research samples was determined by the ethical utilization of experimental animals in the health-care sector using the principle of replacement, reduction, and refinement. The research was conducted after obtaining the recommendation of ethical clearance from the Medical Research Ethics Committee of Hasanuddin University Faculty of Medicine with Recommendation Letter of Ethical Approval no. 1336/H4.8.4.5.31/PP36-KOMETIK/2016 on October 28, 2016. The study was conducted at the Laboratory of Molecular Microbiology and Immunology Faculty of Medicine, Hasanuddin University, Makassar, at the end of November 2016 until early December 2016.

Twenty adult male BALB/c mice were divided into the following two groups: lidocaine and control. Each group consisted of ten BALB/c mice. A blood sample (0.3 ml) was taken from the tail vein of each mouse for the examination of initial HMGB1 mRNA expression and protein levels (first blood test). The mice were then anesthetized with 50 mg/kg of ketamine, intraperitoneally. The closed fracture musculoskeletal injury was established by breaking the left thigh bone using two needle holders without laceration. Four hours later, the mice underwent the closed fracture; 0.3 ml of blood was taken from the tail vein (second blood test). The lidocaine group was then treated with 2 mg/kg of lidocaine (2% lidocaine, PT Kimia Farma, Jakarta, Indonesia) through tail vein injection, once every 2 h continuously for 24 h. The control group was treated with the same volume of distilled water instead of lidocaine. Two hours after completion of the lidocaine and distilled water administrations, 0.3 ml of blood was drawn from the tail vein of both the lidocaine and control group (third blood test). All blood samples were mixed with L6 solution, processed into nucleic acid extracts, and stored at −80°C before enzyme-linked immunosorbent assay (ELISA) and quantitative real-time polymerase chain reaction (qPCR) examination.

Enzyme-linked immunosorbent assay

The level of HMGB1 protein in the serum was determined with ELISA kits (LifeSpan Bioscience, Inc., Seattle, North America) according to the manuals from the manufacturer.

Quantitative real-time polymerase chain reaction analysis

The qPCR examination was performed using a PCR (Bio-Rad CFX Connect, USA) machine. A mixture of 22.5 µl of PCR Mastermix and SYBR Green QRT was prepared. The following forward and reverse primers (1 µl each) for HMGB1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a 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 PCR mix + primary. First stage amplification was performed at 94°C for 2 s and continued until 40 cycles each for 60 s at 94°C and 45 s at 57°C. The PCR results were analyzed using Bio-Rad CFX Manager 3.1 software (Biorad, USA).

Statistical analysis

The data were processed using SPSS software version 20 (IBM, Armonk, NY, USA). The normally distributed data were compared with a Kolmogorov–Smirnov test and presented as mean ± standard deviation. Comparison of data between groups was made using *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

The mean weights of BALB/c mice in the lidocaine and control groups were 41.3 g and 39.34 g, respectively. There was no significant difference between the two experimental groups.

High-mobility group box 1 mRNA expression

The initial expression of HMGB1 mRNA in the lidocaine group was 6.73 ± 0.66. Four hours after closed fracture musculoskeletal injury, expression increased to 11.90 ± 0.52, and 2 h after systemic lidocaine treatment, the expression decreased to 6.94 ± 0.51 ($P < 0.00$). HMGB1 mRNA expression in the control group was 6.75 ± 0.34. Four hours after closed fracture musculoskeletal injury, expression increased to 11.29 ± 0.64, and 2 h after systemic distilled water administration, the expression increased to 13.49 ± 0.4 ($P > 0.32$) [Figure 1].

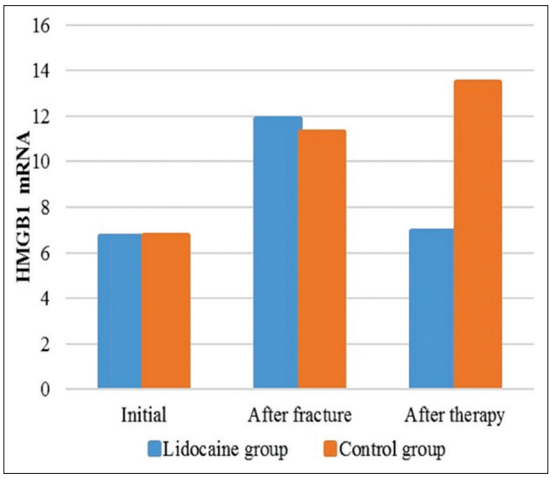

Figure 1: Relative expression of high-mobility group box 1 mRNA of the lidocaine and control groups (n = 10 per group). Data are presented as mean ± standard deviation, $P < 0.05$ compared with control

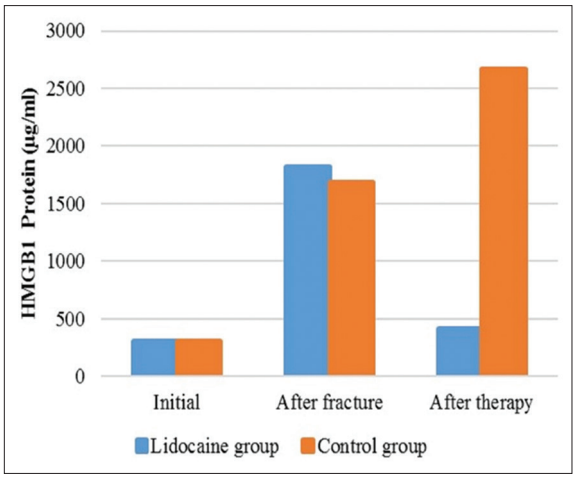

Figure 2: High-mobility group box 1 protein level of the lidocaine and control groups (n = 10 per group). Data are presented as mean ± standard deviation, $P < 0.05$ compared with control

High-mobility group box 1 protein levels

The initial level of HMGB1 protein in the lidocaine group was 306.44 ± 158.54. Four hours after closed fracture musculoskeletal injury, the protein level increased to 1819.88 ± 239.50, and 2 h after systemic lidocaine treatment, the level decreased to 417.00 ± 222.86 ($P < 0.00$). The initial level of HMGB1 protein in the control group was 308.33 ± 188.05. Four hours after the closed fracture injury, the level increased to 1682.67 ± 274.62, and 2 h after systemic distilled water administration, the level increased to 2662.70 ± 269.98 ($P > 0.19$) [Figure 2].

Discussion

HMGB1 is an abundant nonhistone nucleotide protein and can be found in almost all types of mammalian cells. HMGB1 is released passively from necrotic cells and is actively released by macrophage, monocyte, and dendritic cells.[15-17] Excessively increased levels of HMGB1 are associated with worsening of organ function. Surgical stress can stimulate the immune system to produce various cytokines, including HMGB1.[2,18,19]

Our results showed that HMGB1 mRNA expression and protein were present in normal BALB/c mouse blood. Closed fracture increased the expression of HMGB1 mRNA 1.8-fold and protein levels 5.93-fold 4 h after injury. The increased mRNA and protein levels showed that closed fracture musculoskeletal injury inflicted substantial sterile inflammation in the BALB/c mice. After treatment with 2 mg/kg of lidocaine through the tail vein, expression of HMGB1 mRNA decreased from 11.90 ± 0.62 to 6.94 ± 0.51 ($P < 0.00$). Our result shows that intravenous administration of 2 mg/kg lidocaine effectively inhibited transcription of HMGB1 mRNA expression in BALB/c mice with a closed fracture musculoskeletal injury.[20] The increased HMGB1 protein levels decreased from 1819.66 ± 239.50 to 417.00 ± 222.86 after lidocaine therapy for 24 h, showing that intravenous administration of 2 mg/kg of lidocaine was effective in suppressing the translation of HMGB1 protein content in BALB/c mice with a closed fracture musculoskeletal injury.[20-22] The initial HMGB1 protein level was slightly higher than the level after 24 h of systemic lidocaine therapy, but the difference was not statistically significant.

In contrast, the increased expression of HMGB1 mRNA in the control group 4 h after closed fracture continued to significantly increase at 2 h after the 24 h systemic distilled water administration. Likewise, the levels of HMGB1 protein in the control group continued to rise from before the injury to after distilled water administration. The increased levels of control group HMGB1 protein were statistically significant, revealing that systemic distilled water treatment does not effectively suppress sterile inflammation.

The findings of this study show that intravenous administration of 2 mg/kg of lidocaine, once every 2 h continuously for 24 h, was effective to inhibit the transcription of HMGB1 mRNA and suppress the translation of HMGB1 protein in a sterile inflammation model when compared with the control group. The results of this study were consistent with the results of previous research that systematic lidocaine therapy has anti-inflammatory properties in a number of diseases and in animal models of sepsis and organ failure.[21,23-25]

Conclusions

Intravenous administration of 2 mg/kg of lidocaine, once every 2 h continuously for 24 h, effectively inhibited the

inflammatory process in BALB/c mice that underwent closed fracture musculoskeletal injury by suppressing HMGB1 mRNA transcription and HMGB1 protein translation. Our results reemphasize the importance of understanding that systemic lidocaine injection has anti-inflammatory properties in conditions caused by sterile inflammation.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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