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Research article

Lidocaine suppressed hyperinflammation in BALB/c mice model sterile injury via downregulation of toll-like receptor 4

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ABSTRACT

Background: To study the efficacy of systemic lidocaine in suppressing toll-like receptor 4 (TLR4) protein level in BALB/c mouse with sterile injury.
Material and methods: Twenty healthy adult male BALB/c mice were divided into lidocaine and control groups. The sterile injury was performed by breaking the left thigh bone of the mouse without laceration. Four hours after sterile injury the lidocaine group was treated with 2 mg/kg of lidocaine through tail vein injection. The same volume of distilled water was injected into control group instead of lidocaine. Blood was drawn from tail vein before injury, 4 h after sterile injury and 2 h after systemic lidocaine and distilled water administration. TLR4 protein level was examined by enzyme-linked immunosorbent assay (ELISA).
Results: The TLR4 protein level in mice that sustained hyper inflammation due to sterile injury was significantly decreased in the lidocaine group. ($p < 0.00$).
Conclusion: Systemic therapy of lidocaine effectively inhibits TLR4 protein in BALB/c mice that sustained hyperinflammation due to sterile injury.

1. Introduction

Toll-like receptors (TLRs) are identifying receptor initiating innate immune response against substances produced by pathogenic microbes, pathogen-associated molecular patterns (PAMPs) and endogenous molecules released by damaged cells, damage-associate molecular patterns (DAMPs) [1–6]. TLR4 is important to regulate immune system against inflammation caused by infection and trauma [7–11]. Previous studies have shown that when suppressed, TLR4 signaling pathway will provide global protection against sepsis-induced organ dysfunction [12–15]. In addition analgesic and anti-arrhythmia properties, lidocaine also is known to have anti-inflammatory properties and able to modulate inflammatory cascade while possessing protective effect against ischemic injuries on liver, lungs and heart on septic mouse model [16–18]. The anti-inflammatory effect of local anesthesia acts on various cells including monocytes, macrophages and neutrophils. Although lidocaine is important for immune system and inflammation, the mechanisms involved in its action are less understood [19–21]. The aim of this study is to determine whether the injection lidocaine can suppress hyperinflammation response in BALB/c mouse with sterile injury via downregulation of the TLR4 signaling pathway (see Fig. 1).

2. Material and methods

This was a prospective laboratory experimental animal study using 20 healthy adult male BALB/c mice, age 10–12 weeks. Healthy BALB/c mice have glowing eyes no fainted fur, active and have a good appetite. Mice who died during the study were excluded. 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 procedures and principles of the

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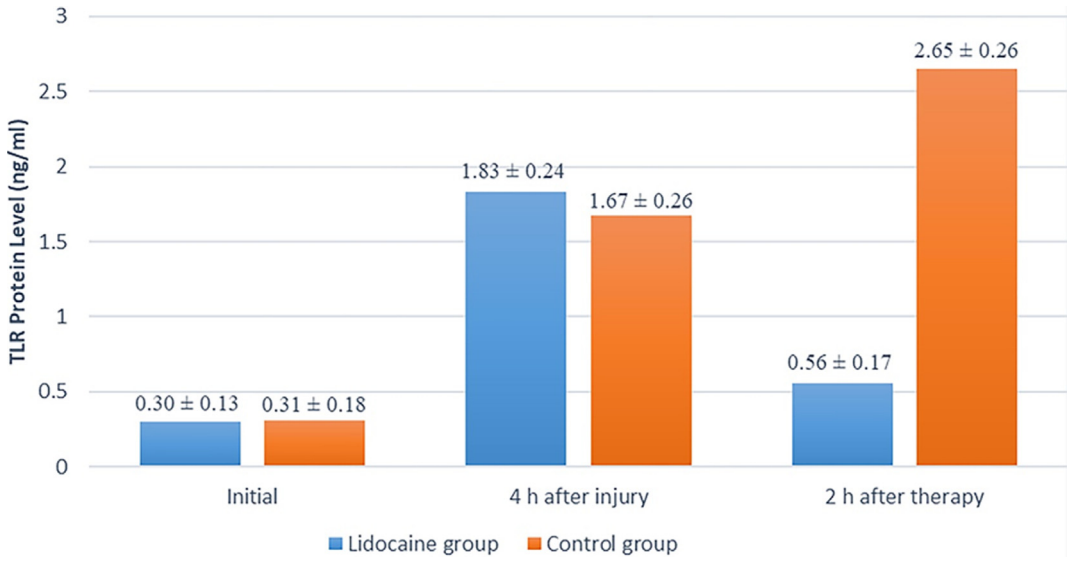

Fig. 1. TLR4 protein level of the lidocaine and control groups (n = 10 per group). Data was presented in form of mean and standard deviation. The p-value was tested with *t*-test and $p < 0.05$ was considered as significant.

Purpose of Control and Supervision of Experiments on Animal (CPCSEA). The number of research samples was determined by the ethical utilization of experimental animals in the healthcare sector using the principle of replacement, reduction and refinement. The research was conducted after obtaining the recommendation of ethical clearance from Medical Research Ethics Committee Faculty of Medicine, Hasanuddin University (Makassar, Indonesia) with registration number UH16050436 dated 28 October 2016. The study was conducted at the Laboratory of Molecular Microbiology and Immunology Faculty of Medicine, Hasanuddin University at the end of November 2016 until early December 2016.

Twenty healthy adult male BALB/c mice were divided into the following two groups: lidocaine and control group. Each group consisted of ten BALB/c mice. A blood sample (0.3 ml) was taken from the tail vein of each mouse for examination of initial TLR4 protein level. The mice were then anesthetized with 50 mg/kg of ketamine, intraperitoneally. A model of sterile injury was established by breaking the left thigh bone using two needle holders without laceration. Four hours after the mice underwent sterile injury, 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 groups (third blood test). All blood samples were collect using centrifugation at 5000 rpm for 5 min and were kept in −80 °C before used.

The level of TLR4 in the serum was determined with ELISA kits (Life Span Bioscience, Inc. Seattle, North America) according to the manuals from the manufacturer.

The data were analyzed using SPSS software version 20. The normally distributed data were tested with Kolmogorov-Smirnov test. The data was then presented as mean ± SD and tested with *t*-test. A value of $p < 0.05$ was considered significant.

3. Results

The mean weights of BALB/c mice in the lidocaine and control groups were 39.30 g and 39.34 g, respectively. There was no significant difference between the two experimental groups ($p > 0.05$).

The initial level of TLR4 protein in the lidocaine group was 0.30 ± 0.13 (ng/ml). Four hours after sterile injury, the protein level increased to 1.83 ± 0.24 (ng/ml), and 2 h after systemic lidocaine treatment, the level decreased to 0.56 ± 0.17 (ng/ml), $p < 0.05$. The initial level of TLR4 protein in the control group 0.31 ± 0.18 (ng/ml). Four hours after the sterile injury, the level increased to 1.67 ± 0.26 (ng/ml), and 2 h after systemic distilled water administration, the level increased to 2.65 ± 0.26 (ng/ml), $p < 0.05$.

4. Discussion

Toll-like receptors are a large family of type I transmembrane protein, function as pattern recognition receptors of the innate immune system [2–4]. TLRs are able to recognize microbes product or pathogen associated molecular patterns (PAMPs) and endogenous ligand related to inflammation or damage associated molecular patterns (DAMPs) [3–5]. TLR4 are extracellular TLRS that first found on mammals, presented mainly by polymorphonuclear leucocytes, monocytes, macrophages, dendritic cells, and any other cells including epithelial and endothelial cells [3,6,7]. TLR4 is important for regulation of immunologic and inflammatory response as it utilized Toll/IL-1 receptor (TIR) domain-containing adapter protein (TIRAP) and MyD88 adapter-like (Mal) to “bridge” myeloid differentiation primary response gene 88 (MyD88) to the receptors and thus activate nuclear factor kappa B (NF-κB). TLR4 transduction signaling used mainly MyD88-dependent pathway, utilizing TIRAP to bridge TLR4 and MyD88 [1,3].

Our research showed that TLR4 protein level were present in normal BALB/c mouse blood. Four hours after sterile injury, the TLR4 protein level increased 6.1 fold in lidocaine group and 5.39 fold in control group. The increased TLR4 protein level showed that sterile injury inflicted substantial sterile hyperinflammation in BALB/c mice. After treatment with 2 mg/kg of lidocaine through the tail vein, once every 2 h continuously for 24 h the level of TLR4 protein decreased from 1.83 ± 0.24 to 0.56 ± 0.15 ($p < 0.00$). Our results showed that intravenous administration of 2 mg/kg of lidocaine, effectively suppressed of TLR4 protein level in BALB/c mouse with a sterile injury [19,20]. In contrast, the level of TLR4 protein in control group continued to rise from before the injury to after distilled water administration. The increased levels of TLR4 protein in control group were statistically significant ($p < 0.05$), revealing that systemic distilled water treatment does not effectively suppress sterile inflammation [20–22].

Previous study showed that systemic lidocaine therapy possessed

anti-inflammatory effect on various diseases or septic model and organ failure on experimental animals via downregulation of TLR4 [3,22,25]. Research conducted by Liu et al. [3], showed that systemic lidocaine therapy can inhibit production of inflammatory mediators including interleukin-6 (IL-6), interleukin-1β (IL-1β), γ interferon, tumor necrosis factor α (TNF α) induced by LPS and down regulation of TLR4 dan NF-κβ [3,25–27]. Activation of NF- κβ is inhibited by systemic lidocaine administration and possess protective effect during sepsis [3].

The finding of this study showed that injection of 2 mg/kg lidocaine, once every 2 h continuously for 24 h effectively suppressed TLR4 protein in sterile hyperinflammation model when compared with the control group. The results of this study were consistent with the results of previous research that systemic lidocaine therapy has anti-inflammatory properties by suppressing hyperinflammation caused by pathogenic infection and sterile injury [20,27].

5. Conclusion

Systemic therapy of 2 mg/kg of lidocaine, once every 2 h continuously for 24 h, effectively suppressed hyperinflammation on BALB/c mouse that underwent sterile injury via downregulation of TLR4 protein level.

6. Author contribution

Following authors have made substantial contribution to the manuscript as under:

Sirait R H: Concept, Data Collection and writing
Hatta M: Review, concept, data analysis
Ramli M: Data analysis, data collection
Siagian C: Bibliography, writing
Suprayogi B: Data collection, data analysis
Simanjutak TP: writing, data collection

Authors agree to be accountable for all aspects of the work in ensuring that question related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of interest

The authors declare there is no conflict of interest.

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Further reading

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