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Original Article

Lidocaine inhibits the production of IL-1β from macrophages RAW264.7 induced with lipopolysaccharide

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Abstract: Uncontrolled inflammation response is a basic section in various diseases, while macrophages play a vital impact in the inflammatory response though producing cytokines. Iterleukin-1β (IL-1β) releasing from activated macrophages is one of the strongest inflammatory cytokines which involved in many inflammatory diseases. Thus, IL-1β represents a potential target for the treatment of inflammation. The traditional local anesthetic lidocaine has demonstrated its anti-inflammatory function in multiple animal disease models and clinical illnesses by suppressing the generation of cytokines. However, it is still unclear if lidocaine could exert the similar effect on IL-1β in vitro. In the present study, we found that lidocaine treatment not only reduced IL-1β in supernatants, but suppressed the mRNA expression of pro-IL-1β and the activation of caspase-1 in RAW264.7 cells induce by LPS. Furthermore, the increased pyroptosis rate and NF-κB activity induced with LPS was also inhibited by lidocaine. Our present study suggest that lidocaine plays the role of antiinflammation by suppressing the generation of IL-1β, and the mechanism might be associated with, at least partly, its inhibitory effects on the signal pathway of inflammasome-NF-κB-caspase-1.

Keywords: Lidocaine, Iterleukin-1β, caspase-1, NF-κB

Introduction

Inflammation is a fundamental section of innate immunity which protects the host from harmful stimuli. But, uncontrolled inflammatory reaction is associated with almost all kinds of diseases. Among the various immune cells, macrophages play a vital impact in the inflammatory response, mainly through phagocytosis, antigen presentation, and cytokine production [1]. Thus, inhibiting the activation of macrophages and its production of cytokines is considered as an important strategy for dealing with inflammatory diseases [2]. Iterleukin-1β (IL-1β) is one of the strongest proinflammatory cytokines releasing from activated macrophages, and it has been demonstrated that IL-1β has a close relation with infection, sterile inflammation, autoimmune disorders [3-6], and even with tumor metastasis [7, 8]. Thus, IL-1β is considered as a feasible target for inflammation treatment. In most cases, caspase-1 cleavage precursor IL-1β (pro-IL-1β) to IL-1β mainly through a nuclear factor-κB (NF-κB)-dependent pathway [9, 10]. Studies indicated when macrophages are treated with LPS, NF-κB is activated and then induced the overexpression of NLRP3 and pro-1L-1β, following by assembly of NLRP3 inflammasome components including caspase-1 [11]. So, NF-κB is the major control point in the product of IL-1β in immune cells. Previous study of us [12, 13] and others [14-16] shown that lidocaine, a traditional local anesthetic, suppress the NF-κB activation *in vivo* and *in vitro*. However, the function of lidocaine on IL-1β release from macrophage has not been clearly explored yet. Thus, the purpose of this study was to evaluate whether lidocaine could reduce the production of IL-1β from macrophages line RAW264.7 induced with LPS and detected its possible mechanism.

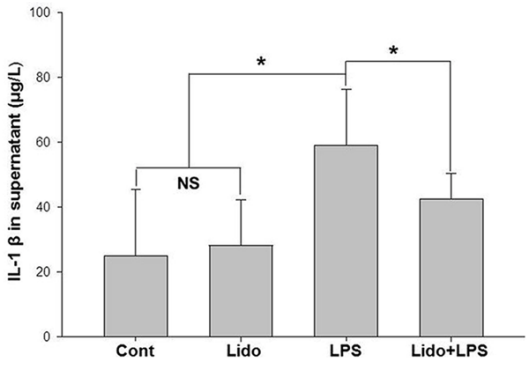

Figure 1. Lidocaine attenuates the release of IL-1β from macrophages. AW264.7 cells were cultured with medium (Cont), lidocaine (Lido, 20 μg/mL), LPS (100 ng/mL) or LPS for 24 h after treated with lidocaine for 4 h (Lido+LPS). IL-1β in supernatants was assayed with ELISA kit. Mean values ± standard deviation (SD). (n=3). *Indicate a significant difference in statistically ($P<0.05$). NS = not significant; Cont = control; Lido = lidocaine; LPS = lipopolysaccharide; IL-1β = Interleukin-1β.

Methods

Cell culture

Macrophages line RAW264.7 cells (ATCC, Rockville, MD, USA) were cultured in complete RPMI1640 medium including 100 ug/mL of streptomycin, 100 U/mL of penicillin, and 10% FBS at 37°C in air containing 5% CO_2. Cells from passages of 4 to 6 were used in the present study.

Cell preparation

RAW 264.7 cells (2.5×10^5 cells/mL) were planted in 6 wells tissue culture dishes and treated with medium alone, lidocaine (20 μg/mL; Zhaohui pharmaceutical Co. LTD., Shanghai, China), LPS (100 ng/mL; Sigma-Aldrich, St. Louis, MO, USA), or LPS for 24 h after treated with lidocaine for 4 h. Cells and supernatants were isolated, collected and stored for tests.

Enzyme-linked immunosorbent assay

IL-1β and caspase-1 in supernatants was quantified with commercial ELISA kits (both from R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

Activity of caspase-1 and nuclear NF-κB p65 assay

Cell proteins were extracted and isolated with nuclear extract kit (Active Motif North America, Carlsbad, CA, USA). Caspase-1 activity in cytoplasm was detected with caspase-1 activity kits (R&D Systems, Minneapolis, MN, USA), and was showed as a relative value. Nuclear NF-κB p65 activation was assayed with TransAM NF-κB kit (Active Motif North America, Carlsbad, CA, USA). All of the above protocols were processed following to the manufacturer's instructions.

Quantitative RT-PCR analysis

Total cells RNA was extracted from treated cells with RNA prep Pure kit (Tiangen Biotech, Beijing, China), and reverse transcription was performed with ReverTra Ace qPCR RT Kit and SYBR Green Real-time PCR Master Mix (both from TOYOBO, Osaka, Japan) following to the manufacturer's protocol. Primers for pro-IL-1β were forward: GAAATGCCACCTTTTGACAGTG, reverse: TGGATGCTCTCAGGACAG; Primers used for caspase-1 were forward: CTTGGAGACATCCTGTCAGGG, reverse: AGTCACAAGACCAGGCATATTCT. Real-time quantitative PCR was performed on a Light Cycler 4.0 Real-Time PCR System (Roche Applied Science, Indianapolis, IN, USA) following PCR program: 30 s at 95°C and then 95°C for 5 s, 57°C for 1 s, and 72°C for 15 s, repeated 40 times. Relative expression values were calculated using Light Cycler 4.0 software.

Flow cytometry

RAW264.7 cells (1×10^5) were labeled with FITC-coupled annexin V and propidium iodide (PI) (annexin V-FITC/PI assay kit; Neobioscience, Shenzhen, China), and quantified with flow cytometry (FACSAria II Flow Cytometer, BD Pharmingen, Oakville, ON, USA) excited at a 488 nm argon ion laser. Cells were sorted according the double-labelled with annexin V-FITC and PI to differentiate healthy ($FITC^-/PI^-$), apoptotic ($FITC^+/PI^-$) and pyroptotic ($FITC^+/PI^+$) cells.

Statistical analysis

Data were expressed as means ± standard deviation (SD). Analysis of treatment effects between groups was performed with a one-way analysis of variance (ANOVA) with Holm-Sidak method for group comparisons using SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA). $P<0.05$ was considered as a statistically significant difference.

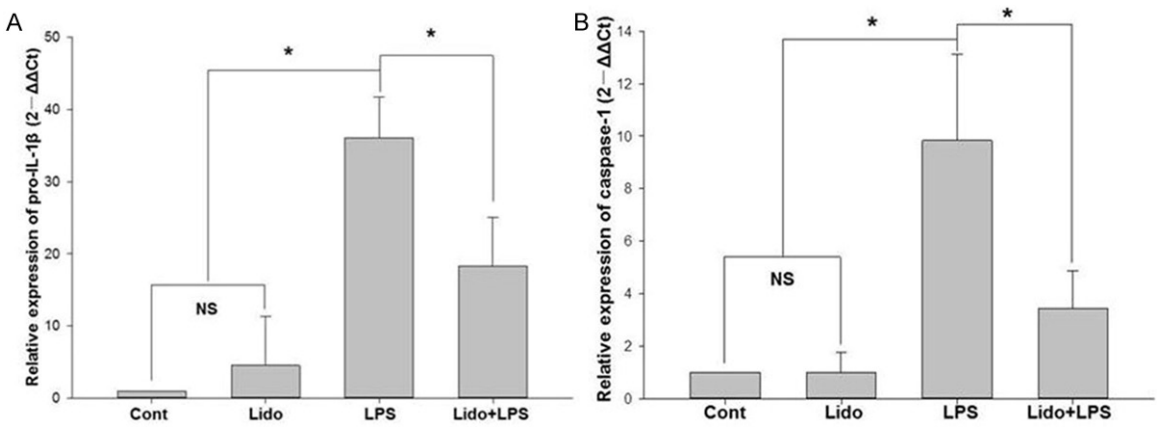

Figure 2. Lidocaine decreases the expression of pro-IL-1β mRNA and caspase-1 mRNA. RAW264.7 cells were cultured with medium (Cont), lidocaine (Lido, 20 μg/mL), LPS (100 ng/mL) or LPS for 24 h after treated with lidocaine for 4 h (Lido+LPS). The mRNA of pro-IL-1β (A) and caspase-1 (B) was assayed with real-time quantitative PCR. Mean values ± standard deviation (SD). (n=14). *Indicate a significant difference in statistically (P<0.05). NS = not significant; Cont = control; Lido = lidocaine; LPS = lipopolysaccharide; Pro-IL-1β = precursor interleukin-1β; mRNA = messenger ribonuclease acid.

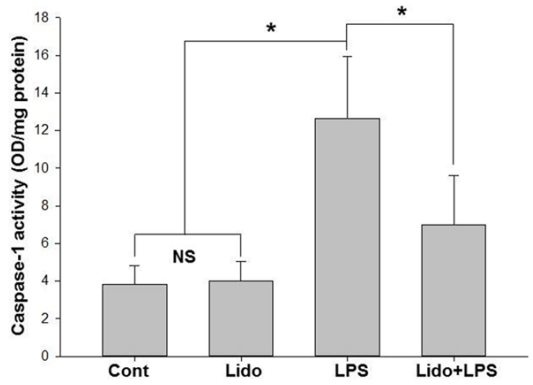

Figure 3. Lidocaine reduces the activity of caspase-1. RAW264.7 cells were cultured with medium (Cont), lidocaine (Lido, 20 μg/mL), LPS (100 ng/mL) or LPS for 24 h after treated with lidocaine for 4 h (Lido+LPS). The cytoplasmic proteins were collected and caspase-1 activity was surveyed using the caspase-1 activity kits. Mean values ± standard deviation (SD). (n=5). *Indicate a significant difference in statistically (P<0.05). NS = not significant; Cont = control; Lido = lidocaine; LPS = lipopolysaccharide.

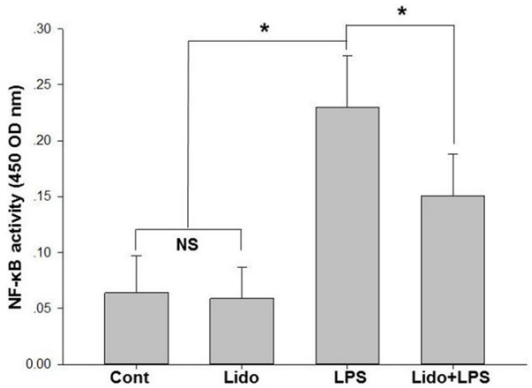

Figure 4. Lidocaine attenuates the activation of NF-κB. RAW264.7 cells were cultured with medium (Cont), lidocaine (Lido, 20 μg/mL), LPS (100 ng/mL) or LPS for 24 h after treated with lidocaine for 4 h (Lido+LPS). The activity of NF-κB was assayed with Active Motif NF-κB family kit. Mean values ± standard deviation (SD). (n=5). *Indicate a significant difference in statistically (P<0.05). NS = not significant; Cont = control; Lido = lidocaine; LPS = lipopolysaccharide; NF-κB = Nuclear factor-κB.

Results

Lidocaine attenuates IL-1β release

To evaluate the effect of lidocaine on the release of IL-1β from macrophages, we treated RAW264.7 cells with lidocaine and then cultured with LPS. Results in **Figure 1** revealed that the level of IL-1β in lidocaine treated group was decreased to compare with that in the LPS group.

Lidocaine decreases the expression of Pro-IL-1β mRNA and caspase-1 mRNA

The increased release of IL-1β from macrophages induced with LPS results from overexpression of pro-IL-1β and its cleavage by caspase-1 [11]. So, we next assayed the mRNA level of pro-IL-1β and caspase-1. Results of RT-PCR showed that lidocaine decreased production of pro-IL-1β and caspase-1 at the transcriptional level (**Figure 2**).

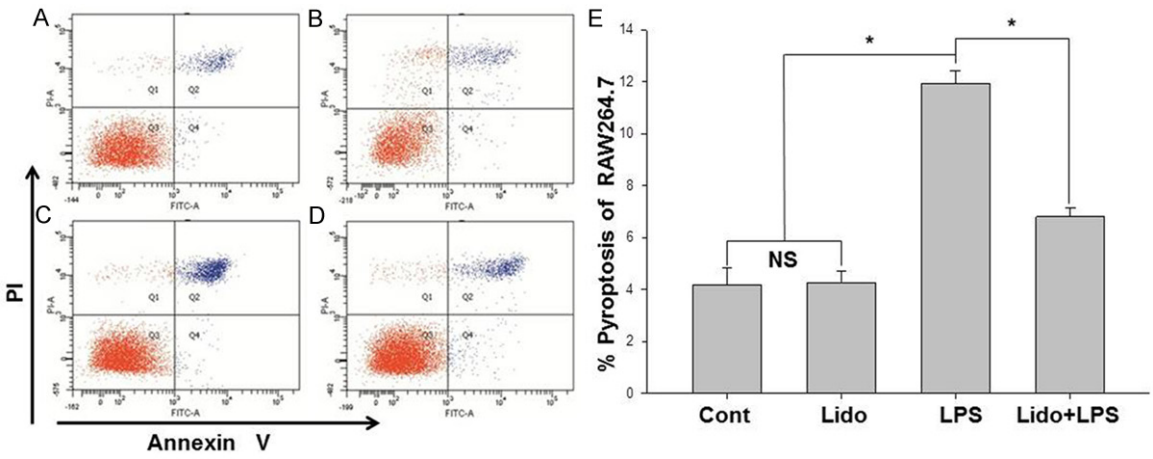

Figure 5. Lidocaine alleviates macrophage pyroptosis. RAW264.7 cells were cultured with medium (A, Cont), lidocaine (B, Lido, 20 μg/mL), LPS (C, 100 ng/mL) or LPS for 24 h after treated with lidocaine for 4 h (D, Lido+LPS). Cells labelled with annexin V-FITC and PI were assayed with Flow Cytometry. Mean values ± standard deviation (SD). (n=5). *Indicate a significant difference in statistically ($P<0.05$). NS = not significant; Cont = control; Lido = lidocaine; LPS = lipopolysaccharide.

Lidocaine reduces caspase-1 activity

To further detect the mechanism in charge of the inhibitory function of lidocaine on the release of IL-1β, we next assayed the impact of lidocaine on the activity of caspase-1. Thus, cytoplasmic proteins were extracted and the caspase-1 activity was observed. Data indicated that lidocaine suppressed the activity of caspase-1 (**Figure 3**).

Lidocaine attenuates the activation of NF-κB

NF-κB activation induced with LPS increases NLRP3 and pro-1L-1β in macrophages [11]. In other words, NF-κB is the upstream molecule which controls the expression of pro-1L-1β and cascase-1. The activity of NF-κB in unclear fraction was decreased in the lidocaine treated group to compare with LPS group as showed in **Figure 4**.

Lidocaine alleviates LPS-induced macrophage pyroptosis

NLRP3 inflammasome signaling cells activate the caspase-1-dependent pyroptotic cell death pathway [17]. Cells double positive labelled with annexin V-FITC and PI were sorted out to pyroptotic macrophages with flow cytometric analysis. Our data suggested that the increased pyroptotic rate of RAW264.7 cells in LPS group was attenuated with lidocaine treatment (**Figure 5**).

Discussion

IL-1β has been set up as a treatment target of infection, sterile inflammation and autoimmune diseases [3] and NF-κB is one of the key controllers of inflammation [13, 15]. Thus, we deduced that medicines which influence on NF-κB activity maybe have broad protection from inflammatory injury. Our present work indicates that lidocaine attenuates the production of IL-1β from LPS-induced macrophage, and this maybe result from its inhibitory effect on the activity of NF-κB and caspase-1. Meanwhile, our data imply that lidocaine might also protect macrophages from pyroptosis, a proinflammatory form of programmed cell death.

Previous studies demonstrated that lidocaine pretreated *in vivo* [18] or treatment delayed *in vitro* [19] reduce the production of IL-1β. In highly developed organisms, IL-1β is generated mostly from macrophages, monocytes, and dendritic cells [20, 21] Studies revealed that when macrophages are stimulated with LPS, NLRP3 inflammasome, NF-κB and caspase-1 are activated in sequence which tightly regulate the maturation of pro-1L-1β to its bioactive form IL-1β [11, 22] and originates the beginning of pyroptosis [23-25]. Meanwhile, inflammasome also mediates HMGB1, a late lethal inflammatory mediator of sepsis and endotoxemia, release from activated immune cells [26, 27]. Our previous study revealed that lidocaine

alleviates the production of HMGB1 and NF-κB activation [13], and the present study of us showed that lidocaine suppress not only the release of IL-1β, but the mRNA expression of pro-IL-1β, the activity of caspase-1 and NF-κB, and the rate of macrophages pyroptosis. Considering of the production of HMGB1 and IL-1β, as well as the macrophages pyroptosis are all mediated in inflammasome-caspase-1 activation dependent manner [26], we infer that the inhibitory effect of lidocaine on cytokines release are mainly regulated by inflammasome- NF-κB -caspase-1 signal pathway.

When pyroptosis occur, macrophages release IL-1β as well as HMGB1 in a caspase-1-dependent manner after pore formation [25, 28]. Although RAW264.7 cells release IL-1β [29] and HMGB1 [30] induced with LPS alone, it is well established as a classical cell pyroptosis model that mouse bone marrow-dervied macrophages stimulated with LPS priming and then with ATP as a second stimulus [29, 31]. Flow cytometric analysis also can't distinguish pyroptoic cells from necrotic when cells labelled with annexin V-FITC and PI [32]. Thus, an elaborately designed protocol, such as using a classical cell pyroptoic model and cells labeled with PI and caspase-1 [33], is needed to find out the direct evidences to elucidate the exact mechanism of lidocaine. The study on microglial cells demonstrated that the protective action of lidocaine on LPS/IFNγ-induced cells injury may be regulated by cell surface ion channel since QX314, a lidocaine analog which can't pass through the plasma membrane, also induces a similar protection [19]. Previous studies demonstrated that blocking of K^+ [34, 35], Na^+ channels [16] are associated with the function of lidocaine on the signaling pathway of NF-κB. So, the upstream signal pathway involved in the inhibitory effect of lidocaine is needed to be illuminated furtherly.

In summary, our current study advises that lidocaine might exert antipyrotic functions by inhibiting the production of IL-1β from immune cells, and which maybe partly result from its suppressing the activation of the inflammasome-NF-κB-caspase-1 signal transduce pathway. The findings in this study provide us a new insight into the anti-inflammatory mechanisms of lidocaine and a novel molecular target for inflammatory diseases treatment.

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Disclosure of conflict of interest

None.

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