

## Original Article

# Intraoperative systemic lidocaine inhibits the expression of HMGB1 in patients undergoing radical hysterectomy

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**Abstract:** Surgery stressors trigger inflammatory response and excessive inflammatory response leads to organ failure or even septic shock. HMGB1 as a later inflammatory cytokines and a critical mediator of severe sepsis is always associated with the aggravation of organ failure. Previous study shows that lidocaine can inhibit the expression of HMGB1 in macrophage of septic rats and protect animals from organ failure. The present study sought to determine whether intraoperative systemic lidocaine could attenuate the level of HMGB1 by inhibiting its expression in PBMC from patients underwent radical hysterectomy. Thirty patients were recruited and divided randomly into two groups according to the difference of study medicine. Patients in lidocaine group received an intravenous bolus infusion of 1.5 mg/kg of lidocaine followed by a continuous infusion of 1.5 mg/kg/h till discharged from operating room, and those in the control group received normal saline. Peripheral blood sample was drawn at pre-surgery, discharge from operating room and 48 h post-surgery. Monocytes were isolated and cultured with medium alone or with LPS. HMGB1 protein in serum or in supernatant of PBMC was detected with ELISA, while the HMGB1 mRNA in PBMC was determined by real-time quantitative PCR. The result showed that lidocaine not only attenuated the level of HMGB1 protein in serum and supernatant, but inhibited the transcription of HMGB1 mRNA in PBMC. The present study of us demonstrated that intraoperative systemic lidocaine can attenuate the level of HMGB1 and inhibit its expression in PBMC from patients underwent radical hysterectomy. Therefore, lidocaine may play an important role in many other clinical diseases by inhibiting HMGB1.

**Keywords:** High mobility group box1, lidocaine, monocytes, gynecologic oncology

## Introduction

Sepsis, defined as a systemic inflammatory response syndrome, always results from larger surgery, serious trauma and infection, and which has been becoming the leading cause of mortality in intensive care units all over the world. Stressors associated with surgery trigger inflammatory response, which play an important role in the healing process; however, an excessive response leads to organ failure [1, 2]. Studies show that inflammatory cytokines such as high mobility group box1 (HMGB1) are always associated with the aggravation of organ failure [3, 4].

HMGB1, a nuclear protein which was widely studied as a transcription factor and growth factor previously, has recently been identified as a critical mediator of severe sepsis [5, 6] and increases in some surgery procedure [7,

8]. Meanwhile, it also contributes to rheumatoid arthritis, colitis, seizures, and liver ischemia/reperfusion injury [9, 10].

Studies show that lidocaine impacts on a number of diseases or disease model by its anti-inflammation effects [11-16]. Data from us reveal that lidocaine can inhibit the expression of HMGB1 in macrophage, decrease the serum level of HMGB1 in septic rats and protect animals from organ failure [17, 18]. But whether lidocaine can exert the same effect on the release of HMGB1 in clinical situation is still unknown.

The present study sought to determine whether intraoperative systemic lidocaine could attenuate the serum level of HMGB1 by inhibiting its expression in monocyte, and ultimately play the role of anti-inflammation in patients underwent radical hysterectomy.

## Lidocaine inhibits HMGB1 expression

**Table 1.** Demographic and clinical characteristics of study participants (mean  $\pm$  SD, n = 15)

Characteristic	Control	Lidocaine	p value
ASA (no.)			
I	10	11	1.00
II	5	4	
Age (yr)	48.6 $\pm$ 5.6	44.2 $\pm$ 11.8	0.20
Height (cm)	156.4 $\pm$ 8.9	155.8 $\pm$ 9.7	0.86
Weight (kg)	64.9 $\pm$ 7.6	60.6 $\pm$ 6.5	0.11
Duration of surgery (min)	129.3 $\pm$ 24.4	132.3 $\pm$ 25.1	0.74
Duration of anesthesia (min)	158.0 $\pm$ 16.9	152.3 $\pm$ 14.1	0.32

### Materials and methods

#### Subjects

Thirty adult female patients (age from 25 to 65 yr) underwent radical hysterectomy were recruited in this prospective study from August 2013 to January 2014. Patients were excluded who weighed less than 45 kg or more than 65 kg; had a history of allergies to local anesthetics; bradycardia, heart block; had severe respiratory, renal or hepatic disease and previous opioids medication or psychiatric medical history.

The study protocol was approved by the Institutional Review Board from Qilu Hospital of Shandong University and carried out according to the Declaration of Helsinki. A written informed consent was obtained from all participants before they were enrolled.

All participants were randomized into two groups according to a random numbers table generated with computer and received intravenous lidocaine or normal saline (control). Study medicines were prepared in a syringe and labeled only with a case number by a nurse who was blind to the protocol. Patients assigned to the lidocaine group received an intravenous bolus infusion of 1.5 mg/kg of lidocaine (Shanghai Zhpharma co.ltd, Shanghai, China) 10 min before anesthesia induction and followed by a continuous infusion of 1.5 mg/kg/h till discharge from operating room. Those in the control group received the same amount of normal saline. The characteristics of patients are shown in **Table 1**.

After administrated with 0.1 mg/kg of midazolam, 1 mg/kg of propofol, 2  $\mu$ g/kg of fentanyl and 0.6 mg/kg of rocuronium intravenously,

patients were intubated and mechanical ventilated to keep the end-tidal CO<sub>2</sub> between 30 and 40 mm Hg. Anesthesia was maintained using 1.5%-3% sevoflurane in 1 L/min oxygen. Fentanyl and rocuronium were added according to heart rate or the bispectral index (BIS) (S/5, GE Healthcare Finland Oy, Helsinki, Finland). Noninvasive arterial blood pressure, electrocardiography and pulse oximetry were monitored continuously. During surgery, the patients received an intravenous infusion of lactated Ringer solution at a rate of 6-12 mL/kg/h.

#### Peripheral blood mononuclear cells (PBMC) isolation

A total of 10 mL of blood sample was drawn from the median cubital vein at the time point of pre-surgery, discharge from operating room and 48 h post-surgery. Serums were isolated at room temperature with centrifugation (1500 r/min, 5 min) and stored -20°C before measurement. PBMC were isolated by Ficollpaque (Haoyang Biological Manufacture co. Lit, Tianjin, China) density centrifugation. The collected cells were washed 3 times with phosphate buffered saline (PBS), resuspended in RPMI 1640 medium (Thermo Fisher Scientific Inc. Shanghai, China) supplemented with 10% heat-inactivated fetal calf serum(FCS), and cultured at 37°C in a humidified 5% CO<sub>2</sub> atmosphere.

#### Cell culture and treatment

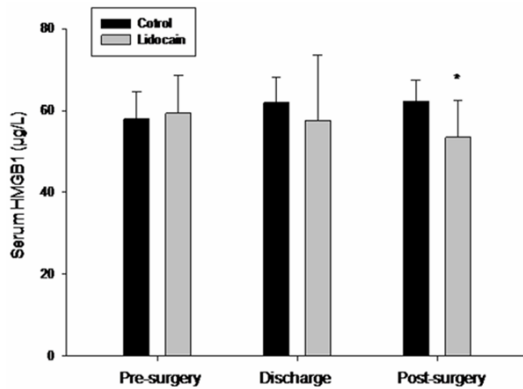
The isolated PBMC were seeded at a density of 1  $\times$  10<sup>6</sup> cells/ml and cultured in RPMI-1640 supplemented with 10% FCS or in RPMI-1640 medium with 10% FCS and LPS (100 ng/ml) at 37°C 5% CO<sub>2</sub> for 24 h. The supernatants from the cell cultures were collected, centrifuged and stored at -20°C for subsequent tests.

Total RNA from 5  $\times$  10<sup>6</sup> PBMC was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol, transferred into eppendorf tube and stored at -80°C.

#### ELISA assay

The level of HMGB1 in the serum and the supernatant of culture medium were determined with

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**Figure 1.** HMGB1 protein level in serum from patients underwent radical hysterectomy. Mean  $\pm$  SD. (n = 15). \*p < 0.05 compared to control.

ELISA kits (R&D, Minneapolis, MN, USA) according to the manuals from the manufacturer.

### Real-time polymerase chain reaction analysis (RT-PCR)

First-strand cDNA was synthesized using 1  $\mu$ g of total cellular RNA as the template, 2  $\mu$ l of 5  $\times$  PrimeScript RT Master Mix, and 7  $\mu$ l of RNase free dH<sub>2</sub>O in a volume of 10  $\mu$ l. Quantitative real-time PCR was performed on a Lightcycler 1536 Real-Time PCR System (Roche Applied Science, Indianapolis, IN, USA). Five microliters of 2  $\times$  SYBR Premix Ex Taq II (Tli RNaseH Plus), 0.8  $\mu$ l of cDNA template, 10  $\mu$ M concentrations of the forward and reverse primers (HMGB1 for, ATA TGG CAA AAG CGG ACA AG; HMGB1 rev, GAC AGG TAA CCA CTA CAA GC.  $\beta$ -actin for, GGC GGC ACC ACC ATG TAC CCT;  $\beta$ -actin rev, TCA TAC TGC TCA GGC CGG GGA), and 3.4  $\mu$ l of dH<sub>2</sub>O in a total volume of 10  $\mu$ l were subjected to the following PCR program: 95°C 30 s, repeated once; 95°C for 5 s, 59°C for 30 s, 60°C for 30 s, repeated 40 times. The product of PCR was evaluated with SYBR Green I and analyzed with Roche Light Cycler 4.0.

### Statistical analysis

SigmaPlot ver. 12.5 (Systat Software, Inc., San Jose, CA, USA) was used for statistical analysis. Data distribution was evaluated using the Levene test. The normally distributed data were presented as mean  $\pm$  SD and compared with the one way ANOVA or unpaired t-test. Descriptive variables were subjected to chi-square analysis or the Fisher exact test, as appropriate. A value of p < 0.05 was considered significant.

## Results

### Preoperative clinical parameters of patients

All of the 30 recruited patients fulfilled the study. As shown in **Table 1**, there were no significant differences between the groups in terms of American Society of Anesthesiologists (ASA) physical status classification, age, height, weight, duration of surgery or anesthesia.

### Lidocaine attenuated the level of HMGB1 protein in serum

HMGB1, as a critical mediator of many inflammation diseases, plays important role in the surgery relative sepsis. To test whether lidocaine has the inhibitory effect on its expression in patients undergoing surgery, the serum level of HMGB1 was first detected with ELISA. In line with our hypothesis, serum HMGB1 protein concentration in lidocaine group attenuated when compared with those in the control group and there was a significantly different at 48 hours after operation (**Figure 1**).

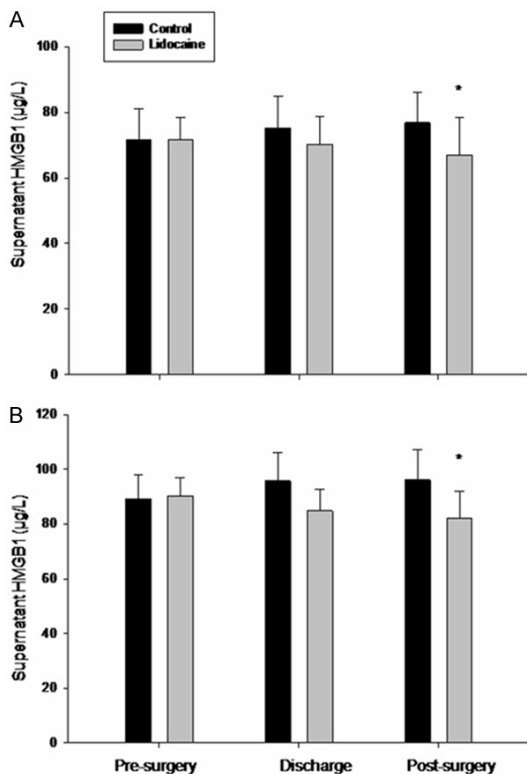
### Lidocaine inhibited the release of HMGB1 in PBMC

The blood monocyte is one of the major contributor to host immunity and to the increase level of HMGB1 protein which involve in the post-operation SIRS, septic shock, and other inflammations. In the present study, PBMC isolated from patients who underwent radical hysterectomy were used to detect the effect of lidocaine on the release of HMGB1. As it showed in **Figure 2A** and **2B**, lidocaine perioperative intravenous administration could attenuate the level of HMGB1 protein in supernatant of monocytes cultured without or with LPS stimulation.

### Lidocaine inhibited the transcription of HMGB1 mRNA in PBMC

To gain further insight into the responsible mechanism for the inhibiting effect of lidocaine on HMGB1 release, we next tested the effect of lidocaine on HMGB1 transcription in PBMC. Thus, the total RNA was extracted from PBMC cultured in media alone or stimulated with LPS and HMGB1 mRNA level was determined by real-time quantitative PCR. Data indicated that lidocaine attenuated HMGB1 production at the transcriptional level (**Figure 3A, 3B**).

## Lidocaine inhibits HMGB1 expression

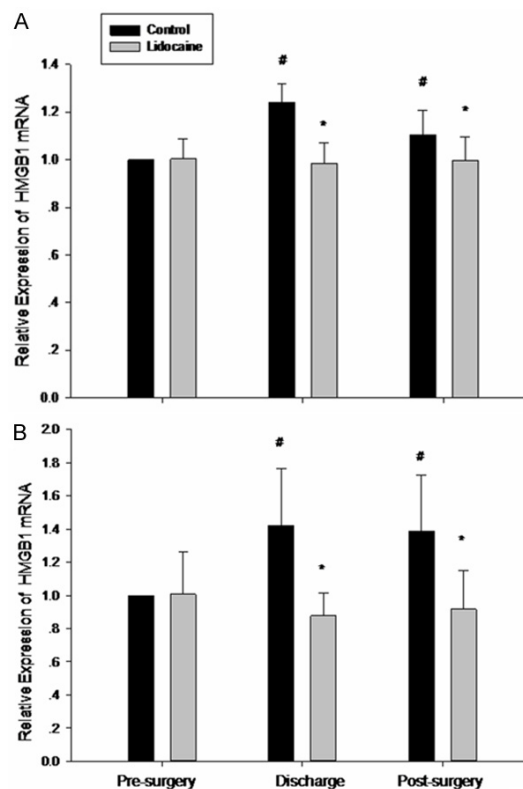


**Figure 2.** HMGB1 protein level in supernatant of PBMC 24 hours after cultured with medium alone (A) or with LPS (100 ng/ml, B). Mean  $\pm$  SD. (n = 15). \*p < 0.05 compared to control.

### Discussion

Lidocaine is commonly used as analgesic and antihyperalgesic in traditional. With the finding of its anti-inflammatory properties, lidocaine has been administrated in some chronic disease such as rheumatoid arthritis, colitis [5, 19]. Recent studies reveal that lidocaine, as an anesthetic adjuvant administrated by perioperative intravenous infusion, benefits in reducing postoperative pain, analgesic consumption, postoperative nausea and vomiting and the length of stay in hospital [20-22]. The present study of us demonstrated that intraperative systemic lidocaine can attenuate the serum leave of HMGB1 and inhibit its expression in PBMC from patients underwent radical hysterectomy.

HMGB1, a non-histone nuclear protein, exist in all kinds of mammalian cell. It can be released passively from necrotic cells or secreted actively by activated immune cells such as macrophages, monocytes and dendritic cells [23]. Unlike the early releasing and disappearing of



**Figure 3.** Relative expression of HMGB1 mRNA in PBMC 24 hours after cultured with medium alone (A) or with LPS (100 ng/ml, B). Data expressed as fold of pre-surgery control groups, Mean  $\pm$  SD. (n = 15). \*p < 0.05 compared to control; #p < 0.05 compared to pre-surgery.

TNF- $\alpha$  and IL-1, the serum concentrations of HMGB1 rise later within 8 to 32 hours after the administration of LPS [5] and maintain high levels for at least 96 hours in animal septic model [24]. Surgical stress stimulates immune system inducing the producing cytokines such as TNF- $\alpha$ , IL-1 and HMGB1. Clinical studies shown that HMGB1 levels increased from the last pringle maneuver to the second day of postoperation in liver resection [7] or from cardiopulmonary bypass detachment to the first postoperative day in cardiac surgery [8]. An increasing of HMGB1 level could be detected with ELISA in this study from the end of surgery to 48 hours after operation in control group. However, lidocaine in clinical relative dose did suppress the produce of HMGB1 induced with surgery stress not only in serum and supernatant, but in the level of transcription.

Surgery stress and the secondary infection are two main causes leading to sepsis. The monocyte/macrophage system is the major contribu-

tor to host immunity and immune surveillance against infection; this may contribute to certain pathological conditions such as SIRS, septic shock, and other inflammations. Our experiments on macrophages and septic animal reveal that lidocaine inhibits the production of HMGB1 in a dose-dependent manner and protects rats from LPS or cecal ligation and puncture assault. Considering of the present result of us and its role of HMGB1 in activation of monocytes [25], induction of adhesion molecules in endothelial cells [26], smooth muscle cell chemotaxis [27], and the development of acute lung injury [28], arthritis [29], and even cancers [20], we suggest that lidocaine may play an important role in treatment for many other clinical diseases by inhibiting production and releasing of HMGB1 under similar pathological conditions. Therefore, our current findings are of importance for understanding the anti-inflammatory effects of lidocaine in sepsis and many other human diseases.

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### Disclosure of conflict of interest

None.

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