

Activated Endothelial Interleukin-1 β , -6, and -8 Concentrations and Intercellular Adhesion Molecule-1 Expression Are Attenuated by Lidocaine

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Endothelial cells play a key role in ischemia reperfusion injury. We investigated the effects of lidocaine on activated human umbilical vein endothelial cell (HUVEC) interleukin (IL)-1 β , IL-6, and IL-8 concentrations and intercellular adhesion molecule-1 (ICAM-1) expression. HUVECs were pretreated with different concentrations of lidocaine (0 to 0.5 mg/mL) for 60 min, thereafter tumor necrosis factor- α was added at a concentration of 2.5 ng/mL and the cells incubated for 4 h. Supernatants were harvested, and cytokine concentrations were analyzed by enzyme-linked immunosorbent assay. Endothelial ICAM-1 expression was analyzed by using flow cytometry. Differences were assessed using analysis of variance and *post hoc* unpaired Student's *t*-test where appropriate. Lidocaine

(0.5 mg/mL) decreased IL-1 β (1.89 ± 0.11 versus 4.16 ± 1.27 pg/mL; $P = 0.009$), IL-6 (65.5 ± 5.14 versus 162 ± 11.5 pg/mL; $P < 0.001$), and IL-8 (3869 ± 785 versus $14,961 \pm 406$ pg/mL; $P < 0.001$) concentrations compared with the control. IL-1 β , IL-6, and IL-8 concentrations in HUVECs treated with clinically relevant plasma concentrations of lidocaine (0.005 mg/mL) were similar to control. ICAM-1 expression on lidocaine-treated (0.05 mg/mL) HUVECs was less than on controls (198 ± 52.7 versus 298 ± 50.3 ; Mean Channel Fluorescence; $P < 0.001$). Activated endothelial IL-1 β , IL-6, and IL-8 concentrations and ICAM-1 expression are attenuated only by lidocaine at concentrations larger than clinically relevant concentrations.

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Ischemia and reperfusion (I/R) injury is a problem that is encountered during a variety of medical and surgical procedures (1). Endothelial cells (ECs) play a key role in I/R injury. They are important producers of and targets for cytokines (2,3), which are associated with neutrophil activation, chemotaxis, neutrophil-EC adhesion, and neutrophil transmigration (4). Tumor necrosis factor (TNF)- α concentrations are increased during I/R injury (4). It results in increased EC production of interleukin (IL)-6, IL-8, and IL-1 β (4) and induces the expression of several adhesion molecules (2), which participate in neutrophil-EC interactions that occur at sites of inflammation and I/R (2).

Local anesthetics are frequently used in the perioperative period and have demonstrated effects on the inflammatory process (5) and I/R injury (6). Lidocaine

decreases cytokine release in epithelial cells and neutrophils (7,8) and attenuates cytokine-induced EC injury (9). Because ECs play a key role in I/R injury, we assessed the effects of lidocaine on activated endothelial cytokine production and intercellular adhesion molecule-1 (ICAM-1) expression.

Methods

Human umbilical vein endothelial cells (HUVECs) from fresh placental cords were isolated by previously described methods (10) and grown until confluence at 37°C in humidified 5% CO₂. The growth medium consisted of complete medium 199 supplemented with 20% fetal calf serum, penicillin (100 U/mL), streptomycin sulfate (100 μ g/mL), Fungizone (0.25 μ g/mL), heparin (16 U/mL), EC growth supplement (75 μ g/mL), and glutamine (2 mM/L). In all experiments, HUVECs were used as individual isolates between passage 3 and 5. At confluence, HUVECs were detached from the culture flask by trypsinization using 0.05% trypsin/0.02% ethylenediamine tetra-acetic acid and seed out on fibronectin-coated

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polycarbonate filters bearing 3.0- μm pores size in Transwell culture plate inserts (Costar, Cambridge, MA). Confluent endothelial monolayers with tight cell junctions were formed after 30 h at 37°C in humidified 5% CO₂ in culture.

The groups were divided as follows: (a) No lidocaine and no TNF- α (normal control); (b) No lidocaine, with TNF- α (experimental control); and (c) Lidocaine, with TNF- α (experimental group).

HUVECs were grown as confluent monolayers in 24-well tissue culture plates (Costar). ECs were used after the cells reached confluence. After culture, the medium was replaced by fresh medium, and then different concentrations of lidocaine (0.005, 0.05, and 0.5 mg/mL) or equal volumes of culture medium 199 were added and incubated for 60 min. Thereafter, TNF- α was added at a concentration of 2.5 ng/mL, and the cells were incubated for 4 h. Viability of cells by trypan blue exclusion was more than 98%.

Subsequently, the supernatants were harvested, and concentrations of IL-1 β , IL-6, or IL-8 were analyzed by enzyme-linked immunosorbent assays (Quantikine R&D Systems Europe Ltd, Abingdon, Oxon, United Kingdom) according to manufacture's instructions. The sensitivity for IL-1 β , IL-6, and IL-8 were 1.0, 0.7, and 10 pg/mL, respectively. The inter- and intra-assay precisions for IL-1 β , IL-6, and IL-8 for the range of values obtained in this study were 2.3%–3.4% and 3.4%–7.1%, 1.7%–4.4% and 2.0%–3.7%, 4.4%–4.7% and 5.2%–8.1%, respectively. Three replicate samples were included in each experiment ($n = 3$).

Stimulated EC suspension 100 μL (1×10^6 cells/mL) was stained with 10 μL of fluorescein-isothiocyanate conjugated anti-CD54 (anti-ICAM-1) mouse anti-human mAb (Becton Dickinson, Oceanside, CA) or 10 μL of fluorescein-isothiocyanate-conjugated isotype immunoglobulin G1 control mAb (Becton Dickinson) and incubated for 30 min at 4°C. ICAM-1 expression on ECs were analyzed on a Fluorescence-Activated Cell-sorter Scanner (FACScan flow cytometry; Becton Dickinson). Three replicate samples were included in each experiment ($n = 3$). The mean channel fluorescence (MCF) intensity of stained cells was detected on the basis of a minimum number of 5000 cells collected and analyzed using the FACScan Research Software version B (Becton Dickinson).

The Sigma Stat 2.0 for windows (SPSS, Inc, Chicago, IL) software package was used for all statistical analysis. Data are reported as mean \pm SD. Differences among groups were assessed by analysis of variance and *post hoc* unpaired Student's *t*-test where appropriate. We used Bonferroni correction for multiple comparisons. A value of $P < 0.0125$ was considered statistically significant.

Results

TNF- α increased HUVEC supernatant IL-1 β concentrations (group B) compared with isolated HUVEC alone

(group A) (4.16 ± 1.27 pg/mL versus 1.15 ± 0.13 pg/mL; $P = 0.007$; Fig. 1). Treatment of HUVECs with lidocaine (0.5 mg/mL but not 0.05 and 0.005 mg/mL) (group C) decreased supernatant IL-1 β concentrations compared with the experimental control (group B; Fig. 1).

TNF- α increased HUVEC supernatant IL-6 concentration (group B) compared with isolated HUVEC alone (group A) (162 ± 11.5 pg/mL versus 37.1 ± 5.28 pg/mL; $P < 0.001$; Fig. 2). Treatment of HUVECs with lidocaine (0.5 mg/mL but not 0.05 and 0.005 mg/mL) (group C) decreased supernatant IL-6 concentrations compared with group B (Fig. 2).

TNF- α increased HUVEC supernatant IL-8 concentrations (group B) compared with isolated HUVEC alone (group A) ($14,961 \pm 406$ pg/mL versus 1596 ± 183 pg/mL; $P < 0.001$; Fig. 3). Treatment of HUVECs with lidocaine (0.5 mg/mL but not 0.05 and 0.005 mg/mL) (group C) decreased supernatant IL-8 concentrations compared with group B (Fig. 3).

TNF- α increased HUVEC ICAM-1 expression (group B) compared with isolated HUVEC alone (group A) (298 ± 50.3 MCF versus 134 ± 14.2 MCF; $P = 0.008$; Fig. 4). Treatment of HUVECs with concentrations of lidocaine (0.05 and 0.5 mg/mL but not 0.005 mg/mL) (group C) decreased ICAM-1 expression compared with group B (Fig. 4).

Discussion

Lidocaine decreased endothelial supernatant IL-1 β , IL-6, and IL-8 concentrations and endothelial ICAM-1 expression when activated in the presence of TNF- α . The effect of lidocaine treatment was concentration dependent. Lidocaine at clinically relevant plasma concentrations (0.005 mg/mL) did not decrease endothelial ICAM-1 expression or cytokine concentrations. However, ICAM-1 expression and cytokine concentrations were decreased at lidocaine concentrations larger than those found in plasma after epidural infusion and IV administration (11).

Hindlimb skeletal muscle injury and remote pulmonary injury are TNF- α and IL-1 dependent (12). During reperfusion after hindlimb ischemia, increased concentrations of TNF- α , IL-1, and IL-6 were found in plasma (13). TNF- α plays a role in the induction of the cytokine network essential to cellular recruitment to the area of injury (14). It also increases expression of ICAM-1 on the vascular endothelium (15,16). ICAM-1 is a counter receptor for neutrophil CD11b/CD18 adhesion molecules. In this study, TNF- α increased endothelial supernatant IL-1 β , IL-6, and IL-8 concentrations and endothelial ICAM-1 expression.

Lidocaine and related local anesthetics inhibit the secretion of proinflammatory cytokines from cultured intestinal epithelial cell lines stimulated by TNF- α (7). The effect of lidocaine on human EC cytokine release

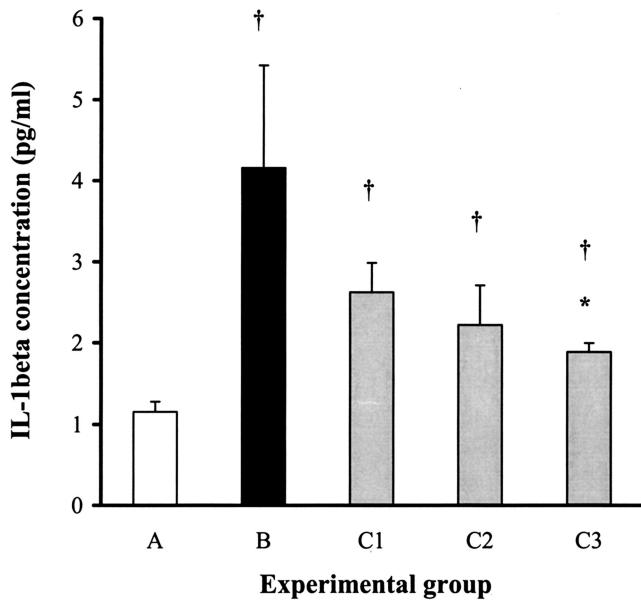


Figure 1. The effect of lidocaine on the endothelial supernatant interleukin(IL)-1 β concentration after stimulation by tumor necrosis factor- α (TNF- α). The experimental groups are represented on the x-axis: A = normal control, human umbilical vein endothelial cells (HUVECs) with culture medium, no TNF- α , and no lidocaine; B = experimental control, HUVECs with TNF- α , and no lidocaine; C1, C2, and C3 = experimental groups, HUVECs with TNF- α , and treatment with 0.005, 0.05, and 0.5 mg/mL of lidocaine, respectively. †*P* < 0.0125 compared with normal control; **P* < 0.0125 compared with experimental control.

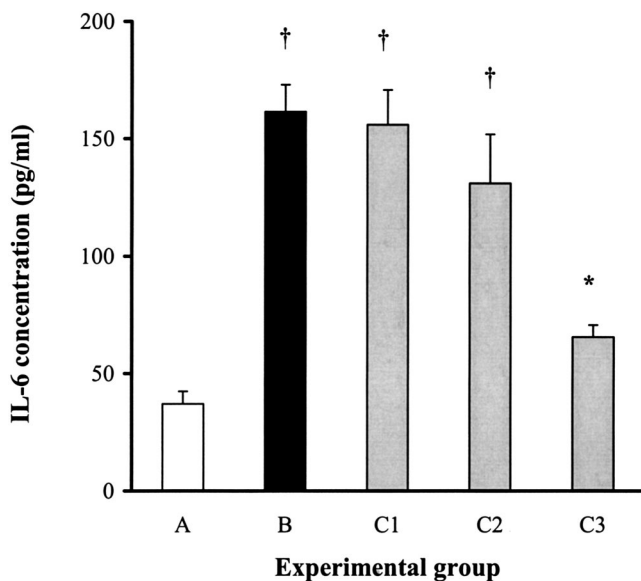


Figure 2. The effect of lidocaine on the endothelial supernatant interleukin(IL)-6 concentration after stimulation by tumor necrosis factor- α (TNF- α). The experimental groups are represented on the x-axis: A = normal control, human umbilical vein endothelial cells (HUVECs) with culture medium, no TNF- α , and no lidocaine; B = experimental control, HUVECs with TNF- α , and no lidocaine; C1, C2, and C3 = experimental groups, HUVECs with TNF- α , and treatment with 0.005, 0.05, and 0.5 mg/mL of lidocaine, respectively. †*P* < 0.0125 compared with normal control; **P* < 0.0125 compared with experimental control.

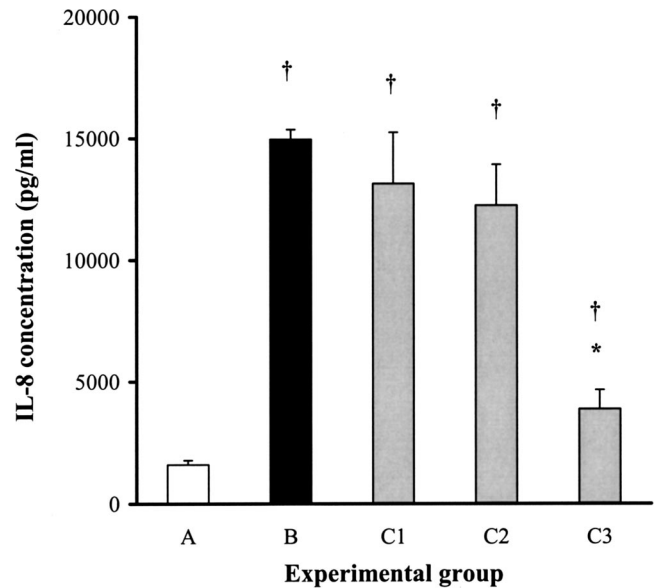


Figure 3. The effect of lidocaine on the endothelial supernatant interleukin(IL)-8 concentration after stimulation by tumor necrosis factor- α (TNF- α). The experimental groups are represented on the x-axis: A = normal control, human umbilical vein endothelial cells (HUVECs) with culture medium, no TNF- α , and no lidocaine; B = experimental control, HUVECs with TNF- α , and no lidocaine; C1, C2, and C3 = experimental groups, HUVECs with TNF- α , and treatment with 0.005, 0.05, and 0.5 mg/mL of lidocaine, respectively. †*P* < 0.0125 compared with normal control; **P* < 0.0125 compared with experimental control.

and adhesion molecule expression has not been studied. These effects are particularly relevant in understanding the possible protective role of local anesthetics in I/R injury. In this study, HUVECs were used to examine the effect of lidocaine on endothelial cytokine release and adhesion molecule expression. HUVECs are the most frequently used EC culture model, although there are few studies comparing their response with other human EC types from the adult organism. Klein et al. (17) have reported similar *in vitro* cytokine effects on the expression of adhesion molecules by HUVEC, saphenous vein, and femoral artery ECs.

Lidocaine, at clinically relevant plasma concentrations (0.005 mg/mL), did not decrease activated HUVEC ICAM-1 expression or supernatant cytokine concentrations. ICAM-1 expression and cytokine concentrations were decreased at lidocaine concentrations of 0.05 mg/mL and 0.5 mg/mL. These concentrations can be achieved in tissues at or near the site of injection (18).

Lidocaine inhibits cytokine-signaling pathways by decreasing protein kinase C activity and protein phosphorylation (19). It also prevents the increase in intracellular Ca²⁺ concentrations during ischemia (20). An increase in free cytosolic Ca²⁺ in ECs is an important early signaling event in neutrophil-endothelial adhesion (21). Lidocaine may thus decrease TNF- α -activated endothelial cytokine production and ICAM-1 expression by inhibiting signals of activation.

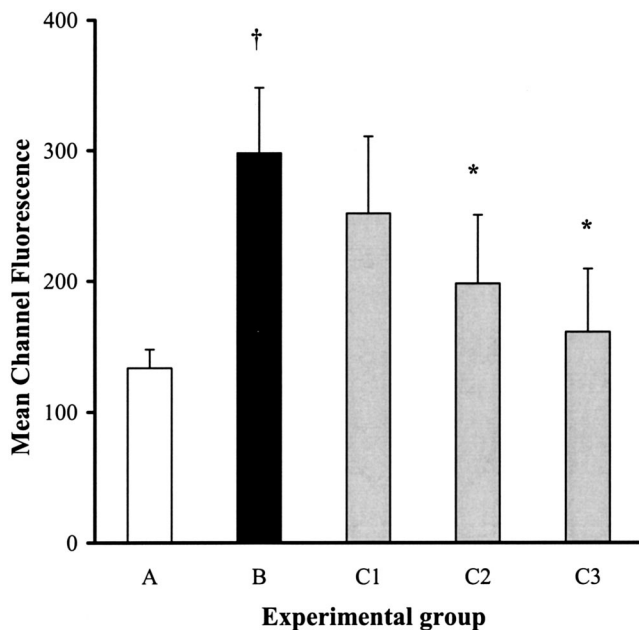


Figure 4. The effect of lidocaine on endothelial intercellular adhesion molecule-1 (ICAM-1) expression after stimulation by tumor necrosis factor- α (TNF- α). The experimental groups are represented on the x-axis: A = normal control, human umbilical vein endothelial cells (HUVECs) with culture medium, no TNF- α , and no lidocaine; B = experimental control, HUVECs with TNF- α , and no lidocaine; C1, C2, and C3 = experimental groups, HUVECs with TNF- α , treatment with 0.005, 0.05, and 0.5 mg/mL of lidocaine, respectively. † $P < 0.0125$ compared with normal control; * $P < 0.0125$ compared with experimental control.

Study limitations include the use of *in vitro* experiments using maximal stimulation and the absence of determination of the mechanism of effect. We did not determine the effect of lidocaine alone (i.e., without agonist) on our assays. It is thus possible that the presence of lidocaine may have interfered with the assays and, thereby, may have resulted in smaller cytokine concentrations and ICAM-1 expression. Taniguchi et al. (22), in an *in vivo* study, demonstrated the absence of a lidocaine effect on cytokine assays. Our study did not investigate different effects of ester and amide local anesthetics. A protective effect in I/R injury by lidocaine concentrations found in tissues at or near the site of injection should be further evaluated.

In conclusion, we have shown that lidocaine at clinically relevant plasma concentrations did not decrease activated endothelial ICAM-1 expression or IL-1 β , IL-6, and IL-8 production but does at larger concentrations.

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