Lidocaine depresses splenocyte immune functions following trauma-hemorrhage in mice

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Kawasaki, Takashi, Mashkoor A. Choudhry, Martin G. Schwacha, Kirby I. Bland, and Irshad H. Chaudry. Lidocaine depresses splenocyte immune functions following trauma-hemorrhage in mice. Am J Physiol Cell Physiol 291: C1049–C1055, 2006. First published June 28, 2006; doi:10.1152/ajpcell.00252.2006.—Traumatic and/or surgical injury as well as hemorrhage induces profound suppression of cellular immunity. Although local anesthetics have been shown to impair immune responses, it remains unclear whether lidocaine affects lymphocyte functions following trauma-hemorrhage (T-H). We hypothesized that lidocaine will potentiate the suppression of lymphocyte functions after T-H. To test this, we randomly assigned male C3H/HeN (6–8 wk) mice to sham operation or T-H. T-H was induced by midline laparotomy and ∼90 min of hemorrhagic shock (blood pressure 35 mmHg), followed by fluid resuscitation (4× shed blood volume in the form of Ringer lactate). Two hours later, the mice were killed and splenocytes and bone marrow cells were isolated. The effects of lidocaine on concanavalin A-stimulated splenocyte proliferation and cytokine production in both sham-operated and T-H mice were assessed. The effects of lidocaine on LPS-stimulated bone marrow cell proliferation and cytokine production were also assessed. The results indicate that T-H suppresses cell proliferation, Th1 cytokine production, and MAPK activation in splenocytes. In contrast, cell proliferation, cytokine production, and MAPK activation in bone marrow cells were significantly higher 2 h after T-H compared with shams. Lidocaine depressed immune responses in splenocytes; however, it had no effect in bone marrow cells in either sham or T-H mice. The enhanced immunosuppressive effects of lidocaine could contribute to the host’s enhanced susceptibility to infection following T-H.

MATERIALS AND METHODS

Traumatic injury due to soft tissue damage and burn induces profound suppression of immune responses (3, 5, 6, 29, 33, 37). Hemorrhage, a commonly encountered complication of traumatic and/or surgical injury, is also known to produce severe depression of cellular immunity (9, 40, 41). A number of studies have demonstrated that splenocyte functions, such as proliferative capacity and cytokine release, and macrophage antigen presentation function are depressed following trauma-hemorrhage (1, 2, 4, 23, 28, 46, 47). The depression of the aforementioned immune functions is associated with increased circulating inflammatory cytokine concentrations, e.g., tumor necrosis factor (TNF) and interleukin (IL)-6. This action leads to an increased susceptibility to sepsis and/or multiple organ failure. In this respect, multiple organ failure is a common clinical problem in surgical intensive care units and is associated with a high mortality rate (47).

Local anesthetics are widely used in patients with trauma and surgical injury for analgesia. They are used to block the conduction of pain through the peripheral and central nervous system. In the intensive care unit, the emergency room, and the operating room, lidocaine is also frequently used as an antiarhythmic agent. Although local anesthetics have been shown to exhibit antibacterial actions as well as beneficial effects on inflammatory response (14, 16, 38), they are also known to have harmful effects on immune function (18, 26, 34, 42). Previous studies have shown that local anesthetics induce neutrophil (18, 34), natural killer cell (42), and monocyte (26) dysfunction. In view of this, local anesthetics have been considered the double-edged sword for the immune response.

It has been demonstrated that local anesthetics modulate inflammatory cascades and have a protective effect on ischemic reperfusion injury in the lung, heart, and liver (16). On the other hand, a report indicates that local anesthetics worsen renal function after ischemic reperfusion injury (43). However, it remains unknown whether local anesthetics have any deleterious or salutary effects on immune cell function following trauma-hemorrhage. The aim of this study, therefore, was to examine the effect of lidocaine on mouse splenocytes and bone marrow cell functions following trauma-hemorrhage.

Mice. Male C3H/HeN mice (Charles River Laboratories, Wilmington, MA), 6–8 wk old and weighing 20–25 g, were used in the experiments. The mice were allowed to acclimatize to the animal facility for 1 wk before the experiments. Animal experiments were conducted in accordance with guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

Trauma-hemorrhage. Animals were anesthetized with isoflurane (Attane; Minrad, Buffalo, NY) and restrained in supine position. A 2.0-cm midline laparotomy (i.e., induction of soft tissue trauma) was performed and then closed aseptically in two layers with 6.0 sutures (Ethilon; Ethicon, Somerville, NJ). Subsequently, both femoral arteries were aseptically catheterized with polyethylene-10 tubing (Clay-Adams, Parsippany, NJ), and the animals were allowed to awaken. Blood pressure was monitored continuously through one of the femoral catheters with a blood pressure analyzer (Digi-Med BPA-190; Micro-Med, Louisville, KY). Upon awakening, the animals were bled through the other catheter to a mean arterial pressure (MAP) of 35 ± 5 mmHg that was maintained for 90 min. At the end of that period, animals were resuscitated with four times the shed blood volume in the form of lactated Ringer solution over 30 min. Lidocaine was applied to the groin incision sites, the catheters were removed, the vessels were ligated, and the incisions were closed with 6.0 sutures. Sham-operated animals underwent the same anesthetic and surgical

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procedures, but neither hemorrhage nor fluid resuscitation was performed. The animals were anesthetized by isoflurane inhalation at 2 h after trauma-hemorrhage, and blood, bone, and spleen were collected for analysis.

Splenocyte preparation. As described previously (47), the spleen was placed in ice-cold, 4°C PBS and gently ground between frosted slides to produce a single-cell suspension. The suspension was centrifuged at 300 g for 15 min, the pellet was resuspended in PBS, erythrocytes were hypotonically lysed, and the remaining cells were washed with PBS by centrifugation at 300 g for 15 min. The splenocytes were resuspended to a concentration of 10^6 cells/ml and cultured in 24-well plates with the use of RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated FBS and 2.5 μg/ml concanavalin A (ConA; Pharmacia/LKB Biotech, Piscataway, NJ) for 48 h at 37°C with 5% CO2 and 95% humidity with or without various concentrations of lidocaine. After the incubation period, cell-free supernatants were harvested and kept frozen at −80°C until assayed.

Bone marrow cell preparation. The bone marrow cells were harvested aseptically from the femoral and tibial bone marrow. The bone marrow cells were collected from each bone. The cells were centrifuged at 400 g at 4°C for 15 min, the supernatant was discarded, and the erythrocytes in the pellets were lysed with sterile water. The bone marrow cells were resuspended in complete RPMI 1640 containing 10% heat-inactivated FBS. The cells were stimulated with 1 μg/ml LPS at 37°C, 5% CO2, and 95% humidity for 48 h with or without various concentrations of lidocaine and centrifuged at 400 g for 10 min, and cell-free supernatants were collected and stored at −80°C for assay.

Cell viability assay. The cell survival assay of splenocytes and bone marrow cells (1×10^6 cells/ml each) was performed up to 5 days in the presence or absence of lidocaine. Cells were stained with Trypan blue, and the percentage of viable cells was calculated by dividing the number of live cells by the total cell number. Change in the percentage of viable cells was calculated on the basis of the number of cells added to the culture on day 0.

Cell proliferation assay. An ELISA kit (Amer sham Biosciences, Piscataway, NJ) was used to determine the proliferation of splenocytes and bone marrow cells. Briefly, splenocytes and bone marrow cells (1×10^6 cells/ml) were plated into a 96-well microtiter plate with or without 2.5 μg/ml ConA (for splenocytes) or 1 μg/ml LPS (for bone marrow cells) stimulation in the various concentrations of lidocaine and cultured at 37°C, 5% CO2, and 95% humidity for 48 h. BrdU was added to the cell suspension to a concentration of 10 μM, and incubation was continued for an additional 8 h for splenocytes and 16 h for bone marrow cells. The plates were washed at 4°C for 10 min, supernatants were carefully removed, and the cells were dried with a hair dryer for 15 min. The cells were incubated with blocking buffer for 30 min at room temperature, after which the blocking buffer was removed by centrifugation. Peroxidase-labeled anti-BrdU reagent was then added, and the plates were incubated for an additional 90 min at room temperature. The plates were washed three times with PBS, and after the addition of 3,3′,5,5′-tetramethylbenzidine and 1 M sulfuric acid, the intensity of the color was measured with a Bio-Tek (PowerWave, Winooski, VT) plate reader.

Detection of phosphorylated (activated) MAPK signaling molecules by fluorescein-activated cell sorting. We measured intracellular signaling molecules using fluorescein-activated cell sorter analysis, as described previously (31). After preparation of splenocytes and bone marrow cells, cells were incubated with lidocaine for 1 h. The cells were then incubated with 2.5 μg/ml ConA (for splenocytes) or 1 μg/ml LPS (for bone marrow cells) for 15 min. After incubation periods, the cells were rapidly fixed with 2% paraformaldehyde for 10 min at 37°C, permeabilized with ice-cold methanol (100%) for an additional 10 min, and washed with PBS supplemented with 1% bovine serum albumin and 0.1% sodium azide. Before antibody staining, samples were incubated with Fc block antibody (eBiosciences, San Diego, CA) to prevent nonspecific binding and then stained with unlabeled primary mouse monoclonal antibodies specific for phosphorylated (active) forms of p38, ERK1/2, or SAPK/JNK (Cell Signaling, Beverly, MA). An isotype-matched mouse IgG was used as a nonspecific staining control. Cells were then washed and stained with a goat anti-mouse FITC-labeled secondary antibody (Jackson ImmunoResearch, West Grove, PA). Cells were subsequently washed twice and resuspended in 0.5% paraformaldehyde. Flow cytometry was performed using the LSR II instrument (BD Biosciences), and the results were analyzed using the accompanying FACSDiva software (BD Biosciences).

Statistical analysis. The data are presented as means ± SE for 6 animals in each group. One-way analysis of variance (ANOVA) and Tukey’s test were used for the comparison between groups, and the differences were considered to be significant at P < 0.05.

RESULTS

Cell viability. Lidocaine (1–100 μg/ml) had no effect on splenocyte and bone marrow cell viability, as determined by the exclusion of the vital stain Trypan blue. However, lidocaine at 1,000 μg/ml decreased cell viability of splenocytes (Fig. 1A) and bone marrow cells (Fig. 1B) from sham and trauma-hemorrhage mice after incubation for 3–5 days. In view of this, subsequent experiments were performed with 100 μg/ml lidocaine.

Splenocyte proliferation. The effect of lidocaine on the ability of splenocytes to proliferate in response to stimulation with ConA in cells from sham and trauma-hemorrhage mice is shown in Fig. 2. The proliferation of splenocytes in response to stimulation with ConA from trauma-hemorrhage mice was depressed compared with sham controls. Lidocaine induced a reduction in splenocyte proliferation from sham controls, and the suppression was greater in splenocytes from trauma-hemorrhage mice incubated with lidocaine than in splenocytes incubated without lidocaine.

Splenocyte cytokine production. The production of Th1 (IL-2 and IFN-γ) cytokines by splenocytes was significantly lower in trauma-hemorrhage mice than in sham controls (Fig. 3). Lidocaine induced a reduction in splenocyte Th1 cytokine production in a dose-dependent manner in both groups. The splenocyte Th2 (IL-4 and IL-10) cytokine production was significantly higher in trauma-hemorrhage mice than in sham controls. Lidocaine induced a decrease in splenocyte IL-4 production in both groups.

Bone marrow cell proliferation. The effect of lidocaine on the ability of bone marrow cells to proliferate in response to stimulation with LPS in sham controls and trauma-hemorrhage mice is shown in Fig. 4. In contrast to splenocyte proliferation, the proliferation of bone marrow cells with LPS stimulation in trauma-hemorrhage mice was increased by ~60% compared with sham mice. However, lidocaine had no effect on bone marrow cell proliferation in either sham controls or trauma-hemorrhage mice. These results indicate that lidocaine did not
affect bone marrow cell functions in terms of bone marrow cell-derived cytokines.

Bone marrow cell cytokine production. The production of TNF-α, MCP-1, and IL-6, but not IL-10, was significantly higher in bone marrow cells from trauma-hemorrhage mice than in sham controls (Fig. 5). However, lidocaine had no effect on bone marrow cell cytokine production in either sham controls or trauma-hemorrhage mice.

MAPK activation. The activation of p38 MAPK and ERK1/2 was significantly lower in splenocytes from trauma-hemorrhage mice than in sham controls (Fig. 6). In contrast, the activation of p38 MAPK and ERK1/2 was significantly higher in bone marrow cells from trauma-hemorrhage mice than in sham controls. Lidocaine (100 μg/ml) induced a suppression of p38 MAPK and ERK1/2 activation in splenocytes in both sham controls and trauma-hemorrhage mice. However, lidocaine had no effect on bone marrow cell MAPK activation in either sham controls or trauma-hemorrhage mice.

DISCUSSION

The results of present study demonstrate that injuries such as soft-tissue trauma and hemorrhage produce profound alterations in immune functions such as cell proliferation and cytokine production in splenocytes and bone marrow cells. The local anesthetic lidocaine further depresses these immune responses in splenocytes; however, it has no effect in bone marrow cells. These results suggest that lidocaine affects T cell immune function, but not bone marrow cell function, in vitro.

We used ConA as a stimulant of lymphocyte proliferation in this study. ConA stimulates the activation of T cells (19). In concurrence with our previous findings (25, 32), there was a significant decrease in the ConA-induced splenocyte proliferation following trauma-hemorrhage compared with the sham control group. In addition, splenocyte Th1 cytokines IL-2 and IFN-γ were decreased in trauma-hemorrhage mice. On the other hand, the release of Th2 cytokines IL-4 and IL-10 was significantly elevated in trauma-hemorrhage mice. Our previous and present results thus suggest that trauma-hemorrhage suppresses T cell proliferation and induces Th2 polarization in splenocytes. Furthermore, the present study demonstrates that Th1 (IL-2 and IFN-γ) cytokine production was inhibited by lidocaine in a dose-dependent manner in vitro. In addition, depressed splenocyte proliferation was further depressed by lidocaine. Because T cell proliferation and differentiation are driven by IL-2 (39), which is produced by the activated T cells, it can be concluded that the decreased IL-2 production induced by trauma-hemorrhage or/and lidocaine is one of the causes of depressed splenocyte proliferation. The activation of p38 MAPK and ERK1/2 was significantly lower in splenocytes from trauma-hemorrhage mice than in sham controls. Lidocaine (100 μg/ml) induced a suppression of p38 MAPK and ERK1/2 activation in splenocyte in both sham controls and trauma-hemorrhage mice. Altogether, our results suggest that the splenocytes immune responses are compromised following trauma-hemorrhage and that the changes in the in vitro immune responses are less affected by lidocaine.
responses become more severe with the addition of lidocaine in vitro.

The response of bone marrow cells is quite different from that of splenocytes following trauma-hemorrhage. The proliferation of bone marrow cells is significantly higher following trauma-hemorrhage compared with the sham control group. In addition, TNF-α, IL-6, and MCP-1 production is significantly higher in mice following trauma-hemorrhage; however, IL-10 production remains unchanged under those conditions. These results are similar to previous findings from our laboratory (25, 32). Furthermore, the higher cytokine production in our studies is also supported by the higher cell proliferation following trauma-hemorrhage. The activation of p38 MAPK and ERK1/2 was significantly higher in bone marrow cells from trauma-hemorrhage mice than in sham controls. Whether these changes in bone marrow cell functions are beneficial or deleterious remain to be determined. In contrast to the splenocytes, lidocaine had no effects on bone marrow cell proliferation and cytokine production. Moreover, lidocaine had no effect on bone marrow cell MAPK activation in either sham controls or trauma-hemorrhage mice. In this study, we used LPS as a stimulant for bone marrow cells because LPS is known to stimulate B cell activation (27). Our group has previously reported that lidocaine has no effect on LPS-induced TNF-α production and on CD14 expression in human monocytes (26). Furthermore, Jinnouchi et al. (21) recently reported that lidocaine and bupivacaine have no inhibitory effect of LPS activation of p38 MAPK in human neutrophils. Therefore, we assume that lidocaine does not affect the LPS signaling pathway in bone marrow cells. These studies collectively suggest that lidocaine affects T cell immune function, but not B cell function, in vitro.

Fig. 3. Effect of lidocaine on splenocyte cytokine production with ConA stimulation. Two hours after T-H, animals were killed, and spleens were aseptically removed and processed for single-cell suspension. Splenocytes were cultured with 2.5 μg/ml ConA for 48 h with or without lidocaine (0–100 μg/ml). Supernatants were harvested for measurements of cytokines IL-2 (A), IFN-γ (B), IL-4 (C), or IL-10 (D). Data are means ± SE from 6 mice in each group. ANOVA: *P < 0.05 compared with equivalent sham; #P < 0.05 compared with no lidocaine addition.

Fig. 4. Effect of lidocaine on proliferation of bone marrow cells with or without LPS stimulation. Two hours after T-H, animals were killed, and bone marrow was aseptically harvested and processed for single cell suspension. Bone marrow cells (1×10^6 cells/ml) were cultured with or without 1 μg/ml LPS and with various concentrations of lidocaine (0–100 μg/ml) for 48 h. Bone marrow cell proliferation was determined using BrdU labeling. Data are means ± SE from 6 mice in each group. ANOVA: *P < 0.05 compared with equivalent sham.
The concentrations of lidocaine used in this study ranged from 1 to 100 µg/ml (4–400 µM). The concentration of lidocaine in human plasma reaches 2.2 µg/ml after epidural administration (8). After 2 mg/ml intravenous administration, the peak plasma concentration of lidocaine reached 1.5–1.9 µg/ml (6–8 µM) within 15 min (45). Similar plasma concentrations were obtained after topical application of lidocaine (1 mg/cm²) in partial thickness burns (7). In this study, the suppressive effects of lidocaine appeared at a higher plasma concentration than is observed in the clinical setting in humans. However, after local application or tissue infiltration of this drug, lidocaine tissue concentration is typically in the millimolar range (17). Therefore, we cannot exclude the possibility that lidocaine adversely affects the immune system when it is administered locally. Nonetheless, it remains to be determined whether subcutaneous administration of lidocaine would also depress immune responses.

The precise mechanism for suppressive effects of lidocaine on splenocyte proliferation and cytokine production remains to be established. The myosin heavy chain (MHC)-II antigen on the mononuclear cell membrane surface is necessary for antigen presentation on the T cell receptor (TCR) on T cells and initiation of immune responses. It has been shown that local anesthetics suppress MHC-II expression on the surface of human monocytes (26). Furthermore, Dickstein et al. (10) reported that lidocaine induced permanent changes on the surface of lymphocytes and reversible changes on the surface of macrophages in vitro. They also reported that chronic exposure to lidocaine resulted in impairment of lymphocyte function in vivo (11). Although the mechanism of the suppressive effect of lidocaine on cell proliferation and cytokine production remains unclear, it is likely that lidocaine impairs the interaction between MHC-II and TCR. This suggestion is based on our results that show that lidocaine has no effects on the proliferation and cytokine production by bone marrow cells.

Both B and T cells originate in the bone marrow; however, only B cells mature there, and T cells migrate to the thymus to undergo their maturation. Although B cells also express MHC-II antigen on their surface, there are no mature T cells that express TCR on their surface in the bone marrow (36). Both B and T cells have the same three major intracellular signaling pathways, which are initiated by cross-linking of lymphocyte receptors (B cell receptors and TCR) by antigen (20). These are MAPK signaling pathway, protein kinase C pathway, and inositol 1,4,5-trisphosphate (IP₃) receptor-mediated intracellular Ca²⁺ release. Our study has demonstrated that lidocaine suppresses activation of MAPK signaling pathway in splenocytes; however, it has no effects on MAPK activation in bone marrow cells. Although we did not investigate the effect of lidocaine on IP₃ receptor-mediated intracellular Ca²⁺ release and protein kinase C activity in splenocytes, previous studies have demonstrated that lidocaine inhibits IP₃ receptor-mediated intracellular Ca²⁺ release (15, 24, 30) and protein kinase C activity (44). Because all of these signaling pathways are upregulated via TCR activation, we believe that the above-mentioned studies support our conclusion of suppressed TCR-dependent pathways. In addition, Gelfand et al. (13) reported that intracellular Ca²⁺ release is critical for IL-2 secretion and cell proliferation of human T lymphocytes. This report also supports our conclusion that lidocaine inhibits IL-2 secretion and splenocyte proliferation via suppression of TCR
activation and the downstream effects of this receptor. Nevertheless, further studies are required to elucidate this mechanism.

One possible limitation of this study is that lidocaine was not administered in vivo, a more physiological environment in which to examine immune functions. Our methods using ex vivo incubation with lidocaine might have increased the confounding factors that may be associated with the isolation of immune cells. In this regard, although in vivo administration is useful for identifying the inhibitory effects of lidocaine on splenocyte function, plasma concentration of lidocaine above 10 μg/ml tends to produce systemic toxicity such as cardiac arrhythmia, hypotension, and cardiovascular collapse (12). In view of those effects, we elected to study the effects of lidocaine in vitro. It also can be argued that we should have examined the effects of other local anesthetics on immune responses. However, because a previous study has shown that neither ropivacaine nor bupivacaine inhibit protein kinase C activity (35), it is possible that both ropivacaine and bupivacaine do not depress splenocyte immune functions. However, it would appear appropriate to investigate the effect of these anesthetics on immune responses in splenocytes, and we hope to conduct those studies in the future. Furthermore, there are other antigen-presenting cells that have MHC-II and TCR interaction, such as Kupffer cells, splenic dendritic cells, and peritoneal and pulmonary macrophages. It is therefore also important to examine the effect of local anesthetics on these cell functions.

In summary, the present study demonstrates that trauma-hemorrhage suppresses cell proliferation and Th1 cytokine production in splenocytes. In contrast, cell proliferation and cytokine production by bone marrow cells were significantly higher 2 h after trauma-hemorrhage compared with sham controls. Whether these changes in bone marrow cell functions are beneficial or deleterious remains the question for future investigation. The observed changes in splenic compartments by lidocaine as shown by significant decreases in the ability of splenic cells to proliferate and to produce Th1 cytokines with an increase in Th2 cytokine production may lead to further immunosuppression following trauma-hemorrhage. Lidocaine impairs immune responses in splenocytes; however, it has no effect in bone marrow cells. The immunosuppressive effects of lidocaine could contribute to the host’s enhanced susceptibility to infection following trauma-hemorrhage. Careful attention must therefore be paid to the choice of local anesthetic and to the effects of lidocaine on host defense mechanisms.

GRANTS
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