Effects of Lidocaine Hydrochloride on Canine Granulocytes, Granulocyte CD11b Expression and Reactive Oxygen Species Production

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ABSTRACT. Lidocaine hydrochloride (Lido) is widely used for analgesia in veterinary medicine; however, in humans, it has been suggested that Lido attenuates granulocyte functions, such as adhesion and reactive oxygen species (ROS) production. Thus, Lido may affect canine granulocyte function; however, there have been no reports on the effects of Lido on canine granulocyte function. Thus, we studied the effects of Lido on canine granulocyte CD11b expression and ROS production. We further studied the effects of Lido on the priming of canine granulocyte CD11b expression and ROS production by recombinant canine granulocyte macrophage colony stimulating factor (rcGM-CSF). Isolated granulocytes were incubated with 3, 30 or 300 μg/ml Lido, or with Lido followed by priming with 5 ng/ml rcGM-CSF. CD11b was detected by the immune fluorescent antibody method, and the mean fluorescence intensity (MFI) was assayed by flow cytometry. ROS production was assessed by the peak time (PT) of ROS production and area under the luminol reaction curve (AUC), which represents total ROS production quantity against opsonized zymosan stimuli. Only 300 μg/ml Lido (tissue level observed by regional block) significantly attenuated both the MFI of CD11b and its enhancement by rcGM-CSF. Moreover, at this concentration, the AUC and its enhancement by rcGM-CSF were significantly attenuated by Lido; in contrast, Lido did not affect PT. In conclusion, Lido suppressed granulocyte adhesion to the endothelium and antiseptic capability by suppressing CD11b expression and/or ROS production. Particular care should thus be exercised when performing regional anesthesia block using Lido.

KEY WORDS: GM-CSF, granulocytes, lidocaine, priming.

Granulocytes, including neutrophils, are often the first cells of the host immune system that encounter invading pathogens such as bacteria and fungi. Granulocyte response to infection is initiated by the adherence of granulocytes to vascular endothelial cells and progresses to the directed migration of granulocytes into the extravascular tissue space. These granulocytes then phagocytize the invading pathogens and kill them through reactive oxygen species (ROS) generation [5].

In inflammatory or infectious conditions in vivo, granulocytes are usually exposed to agents such as inflammatory cytokines or bacterial toxins that exist in the blood or at inflammatory sites [10]. These agents enhance granulocyte function, a response termed priming [1, 10, 25, 26, 32]. These priming components are required for successful host response to tissue injury and infection.

Lidocaine hydrochloride (Lido) is widely used as an analgesic, not only in human medicine but also in veterinary medicine. At clinically recommended doses, Lido has rapid effects, which are of intermediate duration with low toxicity. However, Lido has the potential to suppress granulocyte functions such as CD11b expression, the oxidative burst, phagocytosis and chemotaxis [11, 18, 19, 24]. Lido also attenuates the priming of granulocytes by inflammatory cytokines or bacterial components [14, 23, 31]. The accumulated data concerning the effects of Lido on granulocytes has largely been derived from studies in humans, while there is little information on the effects of Lido on canine granulocyte function or granulocyte priming. Thus, studies on the effects of Lido on canine granulocyte functions are needed.

In this study, we first determined the effects of Lido over a clinically relevant concentration range of 3, 30 or 300 μg/ml on CD11b expression and ROS production in quiescent canine granulocytes. Granulocyte function is mainly originated by recognizing invading agents via granulocyte surface receptors [2, 4, 27]. Complement receptor 3 (CR3) is one such receptor, comprising a heterodimer of CD11b and CD18 that recognizes opsonized particles, indicating that CD11b expression is a good marker of cell activation [28]. Furthermore, the oxidative burst is one of the most important defense mechanisms against infectious diseases and can be stimulated by chemotactants [6, 8]. The priming effects of cytokines are primarily reflected in CD11b expression and the oxidative burst. We thus further studied the effects of Lido (at 3, 30 or 300 μg/ml) on granulocyte priming by recombinant canine granulocyte macrophage colony stimulating factor (rcGM-CSF) based on CD11b expression and ROS production.

MATERIALS AND METHODS

Granulocyte isolation: Granulocytes were isolated from the venous blood of 12 healthy beagle dogs (7 males and 5 females, aged 2 to 7 years). Heparinized blood was mixed 4:1 with phosphate buffered saline, without Ca2+ or Mg2+ [(PBS(–); Sigma Chemical, St. Louis, MO, U.S.A.)], con-

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taining 6.0% dextran T-500 (Wako Pure Chemical Industries, Osaka, Japan) and incubated for 40 min at room temperature to sediment erythrocytes. The leukocyte-rich supernatant was mixed with an equal volume of PBS(−), layered on 15 ml Histopaque-1077 (Sigma Chemical) and centrifuged at 400 x g for 30 min to separate granulocytes from peripheral blood mononuclear cells (PBMCs). Following aspiration of the PBMC layer and remaining supernatant, erythrocytes were lysed with NH4Cl lysis buffer (pH 7.4) for 10 min and centrifuged at 400 x g for 5 min. This procedure yielded a preparation that was composed of >90% granulocytes, >99% of which were viable, as determined by trypan blue dye staining.

The purified granulocyte pellet was resuspended in Hanks’ Balanced Salt Solution, without Ca2+ or Mg2+ (HBSS(−); Gibco, Grand Island, NY, U.S.A.), containing 10% autologous plasma buffered with 10 mM HEPES (pH 7.4) and was used to assess CD11b expression. To assess ROS production, granulocytes were resuspended in HBSS(−) containing 1% fetal bovine serum (FBS; Sanko-Junyaku, Tokyo, Japan) buffered with 10 mM HEPES (pH 7.4).

Granulocyte culture: Suspended granulocytes were treated with Lido (Wako Pure Chemical Industries) at concentrations of 3, 30 or 300 μg/ml at 37°C under 5% CO2 for 30 min. Treatment with saline was used as a control. In some experiments, following treatment with Lido, granulocytes were primed with 5 μg/ml rcGM-CSF (R&D Systems, Minneapolis, MN, U.S.A.) at 37°C under 5% CO2 for 15 min. Pretreatment with saline, followed by priming with rcGM-CSF, was used as the GM-CSF control (G cont), and priming with PBS(−) was used as the priming control (P cont).

Assay of CD11b expression: Granulocytes were incubated with the mouse anti-dog CD11b monoclonal antibody (AbD Serotec, MorphoSys, U.K.) at 4°C for 40 min. To detect antibody-bound cells, cells were then incubated with R. phocoerythrin (RPE)-labeled goat anti-mouse IgG1 (AbD Serotec, MorphoSys, Oxford, U.K.) at 4°C for 40 min. After washing with PBS(−), cells were analyzed by flow cytometry using the Expo 2000 software (Beckman Coulter, Fullerton, CA, U.S.A.). The granulocyte population was gated from residual PBMCs based on forward and side scatter characteristics on dot plots. The geometric mean fluorescent intensity (MFI) on histogram analysis was calculated as the CD11b-expression index and was expressed as a percentage vs. control samples (100%). In this analysis, MFI values indicate the level of CD11b expression.

Preparation of serum-opsonized zymosan (sOZ): Zymosan (Zymosan A from S. cerevisiae, Sigma Chemical) was boiled in ultra-pure water for 60 min, and then washed with sterilized ultra-pure water. After centrifugation, zymosan pellets were resuspended in fresh autologous serum at a concentration of 10 mg/ml and then incubated for 60 min at 37°C. After incubation, the suspension was washed twice with HBSS(−) and re-suspended in HBSS(−) at a concentration of 10 mg/ml.

Oxidative burst assay: Luminol-dependent chemiluminescence (CL) assays were performed with a slight modification of previously established methods [20, 39]. Granulocytes in a solution of HBSS(−) supplemented with 0.5 mM CaCl2 and 1 mM MgCl2, 10 μM luminol (Fluka Chemika Biochemika, Buchs, Switzerland) and 50 μg/ml horseradish peroxidase (Sigma Chemical) were placed at a density of 5 x 106 cells per well in a 96-well microplate (Sumilon, Tokyo, Japan). The cell suspension (315 μl) was incubated for 5 min at 37°C. After incubation, granulocytes were activated by addition of 35 μl sOZ (10 mg/ml), and CL (RLU/s) was measured at 37°C every 0.2 sec with a luminometer (Dynex Technologies, London, U.K.). The response was recorded over a period of 30 min. CL response was indicated by the peak time (PT; sec), which corresponds to the mean time from the recognition of sOZ to the time of maximum release of ROS from granulocytes. The area under the CL curve (AUC), expressed as a percentage vs. control samples (100%), corresponds to the total ROS produced. The effects of Lido on the granulocyte oxidative burst were assayed by luminol-dependent CL assay following activation of granulocytes by addition of 35 μl of sOZ (10 μg/ml). In this assay, PT represents the rate at which granulocytes react to sOZ, and AUC represents total ROS production over 30 min.

Statistical analyses: All experiments were performed in triplicate, and all data are expressed as means ± SD. For analysis of the effect of Lido on canine granulocytes, statistically significant differences obtained on one-way ANOVA analysis were further tested by Dunnett’s test for post-hoc pairwise comparisons. For analysis of the effects of Lido on rcGM-CSF priming of canine granulocytes, statistically significant differences obtained on one-way ANOVA analysis were further tested by Tukey’s test for post-hoc pairwise comparisons. P values of less than 0.05 were considered to be statistically significant.

RESULTS

Effects of Lido on CD11b expression: As shown in Table 1, treatment with either 3 or 30 μg/ml Lido did not affect the MFI values, which were 99.3 ± 6.6% and 97.4 ± 4.4%, respectively. In contrast, treatment with 300 μg/ml Lido decreased the MFI values (MFI: 85.2 ± 21.0%) compared with the controls. The significant difference between the MFI values of the Lido treatment and control indicates that treatment with 300 μg/ml Lido impairs CD11b expression.

Effects of Lido on ROS production: Figure 1 shows the CL value curve, and Table 2 shows the calculated PT and AUC. Treatment with Lido tended to prolong PT at all concentrations tested (623.5 ± 78.9 sec for 3 μg/ml; 623.5 ± 83.0 sec for 30 μg/ml; and 623.5 ± 78.9 sec for 300 μg/ml) compared with the PT in the controls (609.2 ± 77.2 sec); however, the differences observed were not statistically significant. Treatment with low concentrations of Lido did not affect the rate at which granulocytes reacted to sOZ. In addition, after treatment with 3 or 30 μg/ml Lido, the AUC did not show any significant differences (105.0 ± 8.4% for 3
Effects of Lido on canine granulocytes were pretreated with various concentrations of lidocaine (3, 30 and 300 µg/ml) at 37°C under 5% CO2 for 30 min. The mean fluorescence intensity (MFI) of the CD11b expression and polymerized actin are presented as percent vs. control (100%). All data are expressed as means ± SD of three experiments. a) Significant difference vs. control (P<0.05).

Table 1. Effects of lidocaine on CD11b expression

<table>
<thead>
<tr>
<th>Lidocaine concentration</th>
<th>Control</th>
<th>3 µg/ml</th>
<th>30 µg/ml</th>
<th>300 µg/ml</th>
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<tr>
<td>CD11b expression (%)</td>
<td>100</td>
<td>99.3 ± 6.6</td>
<td>97.4 ± 4.4</td>
<td>85.2 ± 21.0</td>
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</table>

Canine granulocytes were pretreated with various concentrations of lidocaine (3, 30 and 300 µg/ml) at 37°C under 5% CO2 for 30 min. The mean fluorescence intensity (MFI) of the CD11b expression and polymerized actin are presented as percent vs. control (100%). All data are expressed as means ± SD of three experiments. a) Significant difference vs. control (P<0.05).

Fig. 1. Time-course effects of lidocaine on chemiluminescence (CL) in canine granulocytes treated with serum opsonized zymosan (sOZ). [] control granulocytes activated with sOZ; ▲ granulocytes pretreated with 3 µg/ml lidocaine before sOZ activation; ■ granulocytes pretreated with 30 µg/ml lidocaine before sOZ activation; ● granulocytes pretreated with 300 µg/ml lidocaine before sOZ activation. Each point for chemiluminescence indicates the mean of three experiments. The standard deviation is omitted.

Table 2. Effects of lidocaine on peak time and ROS production in canine granulocytes

<table>
<thead>
<tr>
<th>Lidocaine concentration</th>
<th>Control</th>
<th>3 µg/ml</th>
<th>30 µg/ml</th>
<th>300 µg/ml</th>
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</thead>
<tbody>
<tr>
<td>Peak time (sec)</td>
<td>609.2 ± 77.2</td>
<td>623.5 ± 78.9</td>
<td>623.5 ± 83.0</td>
<td>623.5 ± 78.9</td>
</tr>
<tr>
<td>AUC (%)</td>
<td>100</td>
<td>105.0 ± 8.4</td>
<td>99.5 ± 4.8</td>
<td>85.3 ± 6.5</td>
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</table>

Canine granulocytes were pretreated with various concentrations of lidocaine (3, 30, 300 µg/ml) at 37°C under 5% CO2 for 30 min. The time point at which the maximal CL value was observed is expressed as the peak time (PT: sec). The area under the CL curve (AUC) was estimated as the total ROS production of canine granulocytes for 30 min and is expressed as percent vs. control (100%). Lidocaine did not affect PT, but ROS production was impaired by 300 µg/ml lidocaine. All data are expressed as means ± SD of three experiments. a) Significant difference vs. control (P<0.05).

Effects of Lido on rcGM-CSF induced up-regulation of CD11b: We analyzed the effects of Lido on up-regulation of CD11b induced by rcGM-CSF using flow cytometry. The calculated MFI, an index of CD11b expression, was increased by rcGM-CSF (164.0 ± 22.1%) compared with the P cont (100%); Table 3. Priming with rcGM-CSF following pretreatment with 3 or 30 µg/ml Lido also increased the MFI of CD11b (164.2 ± 24.4% and 163.2 ± 24.6%, respectively). However, priming with rcGM-CSF following pretreatment
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Table 3. Effects of lidocaine on rcGM-CSF-primed canine granulocytes: CD11b expression

<table>
<thead>
<tr>
<th>Pretreated with saline</th>
<th>Pretreated with lidocaine (Lidocaine concentration)</th>
<th>CD11b expression (%)</th>
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<tbody>
<tr>
<td></td>
<td>GL3 (3 μg/ml)</td>
<td>164.0 ± 22.1a)</td>
</tr>
<tr>
<td></td>
<td>GL30 (30 μg/ml)</td>
<td>164.2 ± 24.4a)</td>
</tr>
<tr>
<td></td>
<td>GL300 (300 μg/ml)</td>
<td>163.2 ± 24.6a)</td>
</tr>
<tr>
<td>P cont</td>
<td>G cont</td>
<td>100</td>
</tr>
<tr>
<td>GL3</td>
<td>GL30</td>
<td>139.9 ± 20.3a,b)</td>
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<tr>
<td>GL30</td>
<td>GL300</td>
<td>123 ± 8.8%</td>
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</table>

Canine granulocytes were pretreated with various concentrations of lidocaine (3, 30, 300 μg/ml) at 37°C under 5% CO2 for 30 min and were primed with rcGM-CSF (5 ng/ml) for 15 min. The mean fluorescence intensity (MFI) of the CD11b expression is presented as percent vs. control (100%). Only 300 μg/ml lidocaine attenuated the up-regulation of CD11b expression by rcGM-CSF. All data are expressed as means ± SD of three experiments. P cont, granulocytes pretreated with saline and primed with PBS(–). G cont, granulocytes pretreated with saline and primed with 5 ng/ml rcGM-CSF. GL3, granulocytes pretreated with 3 μg/ml lidocaine and primed with 5 ng/ml rcGM-CSF; GL30, granulocytes pretreated with 30 μg/ml lidocaine and primed with 5 ng/ml rcGM-CSF; GL300, granulocytes pretreated with 300 μg/ml lidocaine and primed with 5 ng/ml rcGM-CSF. a) Significant difference vs. control (P<0.05); b) Significant difference vs. G cont (P<0.05).

Fig. 2. Time-course effects of lidocaine on rcGM-CSF-primed canine granulocytes: chemiluminescence (CL) with serum opsonized zymosan (sOZ). , granulocytes primed with PBS(–) (P cont) activated with sOZ; , granulocytes primed with recombinant canine GM-CSF (G cont) and activated with sOZ; , granulocytes pretreated with 3 μg/ml lidocaine and primed with rcGM-CSF before sOZ activation (GL3); , granulocytes pretreated with 30 μg/ml lidocaine and primed with rcGM-CSF before sOZ activation (GL30); , granulocytes pretreated with 300 μg/ml lidocaine and primed with rcGM-CSF before sOZ activation (GL300). Each point of chemiluminescence indicates the mean of three experiments. The standard deviation is omitted.

with 300 μg/ml Lido only slightly enhanced the MFI of CD11b (139.9 ± 20.3%) compared with the G cont and with the values obtained following treatment with 3 or 30 μg/ml Lido. Thus, pretreatment with 300 μg/ml Lido inhibited the up-regulation of CD11b expression induced by rcGM-CSF.

Effects of Lido on rcGM-CSF-induced enhancement of the oxidative burst: The effects of Lido pretreatment on rcGM-CSF-enhancement of the oxidative burst were also determined by CL assay. Figure 2 shows the CL value curve, and Table 4 shows the calculated PT and AUC, which represent the rate at which granulocytes react to sOZ.

The priming effects of rcGM-CSF were first confirmed by comparison of the rate of ROS production in granulocytes primed with rcGM-CSF or control PBS(–) followed by activation with sOZ. As shown in Fig. 2 and Table 4, in the absence of Lido pretreatment (G cont), rcGM-CSF priming shortened PT (438.4 ± 61.1 sec) compared with the P cont (585.0 ± 84.4 sec). Pretreatment with Lido prior to priming with rcGM-CSF also shortened PT compared with the controls. The values of PT under these conditions were 432.5 ± 65.5 sec for 3 μg/ml Lido, 440.0 ± 59.8 sec for 30 μg/ml Lido and 467.5 ± 48.6 sec for 300 μg/ml Lido. These results indicate that Lido does not affect the rate at which granulocytes react with sOZ when primed by rcGM-CSF. When the effects of rcGM-CSF on total ROS production over 30 min were assayed, the G cont was observed to enhance ROS production (112.7 ± 9.7%) compared with the P cont (100%).

Treatment with 3 or 30 μg/ml Lido following rcGM-CSF priming enhanced ROS production (113.8 ± 10.4% for 3 μg/ml Lido; 112.3 ± 8.8% for 30 μg/ml Lido). However, treatment with 300 μg/ml Lido following rcGM-CSF priming...
**DISCUSSION**

There is an increasing need for effective pain control methods in veterinary medicine, and local anesthetics (LAs), such as Lido, are used for perioperative pain management. External otitis affects chondrodystrophoid breeds and is a very common surgical procedure in dogs, and Carpenter et al. reported that intrapetitoneal injection of Lido and bupivacaine reduces post-operative pain [9]. Epidural anesthesia is also potently affected by CD11b expression levels. In these instances, granulocytes in the blood stream or in tissues near the injection sites are exposed to various concentrations of Lido.

In the present study, we demonstrated the effects of therapeutic concentrations of Lido on canine granulocyte function in vitro. The 3 μg/ml dose of Lido corresponds to serum levels after intravenous or epidural infusion [30]. As described above, like other LAs, Lido may be injected locally for regional blocks around surgical wounds. LA concentrations at specific sites vary widely, depending on the method of administration, although there have been no reports on LA concentrations in critical tissue after local application or tissue infiltration of these drugs. However, Holst et al. performed a study using an in vitro model of the human spinal canal [22] and examined Lido concentrations in the inter-vertebral spaces after injection of 5% Lido (2.5 ml). Concentrations of 4 mg/ml (approximately 16 mM) were subsequently observed in several segments. Thus, granulocytes could be exposed to concentrations of Lido higher than 300 μg/ml (approximately 1.2 mM) Lido after local application, such as in the case of brachial plexus block and epidural infusion.

We used the surface receptor CD11b as a marker of activated granulocytes. Down-regulation of CD11b indicates granulocyte dysfunction and is observed in immune deficiencies, such as leukocyte adhesion deficiency syndrome (LAD) [3, 12, 35]. Kiefer et al. previously reported the effects of Lido on human granulocytes [24]. They found that the most pronounced local anesthetic effect of Lido was on CD11b expression and that administration of at least 1,846 μM (approximately 432.6 μg/ml) Lido down-regulated CD11b expression. In the present study, 300 μg/ml Lido, but not lower concentrations, down-regulated CD11b expression, which is consistent with data in previous human studies.

The second measure of granulocyte activation that we assayed in this study was the granulocyte oxidative burst induced following recognition of opsonized agents, which is affected by CD11b expression levels. We induced granulocyte ROS production with the agent sOZ. As 300 μg/ml Lido can downregulate CD11b expression, it was predicted that Lido would also inhibit the oxidative burst. Of the doses tested, only 300 μg/ml Lido reduced total ROS production. These results suggest that 300 μg/ml Lido suppresses canine granulocytes mainly by down-regulation of CD11b expression. However, the oxidative burst is regulated by complex internal cell signaling, and there remains a possibility that Lido affects these internal signal transduction pathways. Further study regarding the effects of Lido on the oxidative burst when stimulated by a direct activator of signal transduction, such as phorbol myristate acetate (PMA), is required to clarify this point.

**Table 4. Effects of lidocaine on rcGM-CSF-primed canine granulocytes: ROS production with serum opsonized zymosan (sOZ)**

<table>
<thead>
<tr>
<th>Pretreated with saline</th>
<th>Pretreated with lidocaine (Lidocaine concentration)</th>
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<tr>
<td></td>
<td>P cont</td>
</tr>
<tr>
<td>Peak time (sec)</td>
<td>585.0 ± 84.4</td>
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<tr>
<td>AUC (%)</td>
<td>100</td>
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Canine granulocytes were pretreated with various concentrations of lidocaine (3, 30, 300 μg/ml) at 37°C under 5% CO<sub>2</sub> for 15 min and were primed with rcGM-CSF (5 ng/ml). The time point at which the maximal CL value was observed was estimated as the peak time (PT: sec). The area under the CL curve (AUC) was estimated as the total ROS production of canine granulocytes for 30 min and is expressed as percent vs. control (100%). rcGM-CSF reduced PT, but a priming effect was observed, and none of the concentrations of lidocaine affected the reduction in PT. rcGM-CSF also enhanced ROS production; however, enhancement was not observed with 300 μg/ml lidocaine. All data are expressed as means ± SD of three experiments. P cont, granulocytes pretreated with saline and primed with PBS(−). G cont, granulocytes pretreated with saline and primed with 5 ng/ml rcGM-CSF; GL3, granulocytes pretreated with 3 μg/ml lidocaine and primed with 5 ng/ml rcGM-CSF; GL30, granulocytes pretreated with 30 μg/ml lidocaine and primed with 5 ng/ml rcGM-CSF; GL300, granulocytes pretreated with 300 μg/ml lidocaine and primed with 5 ng/ml rcGM-CSF. a) Significant difference vs. P cont (P<0.05); b) Significant difference vs. G cont (P=0.05).
inflammatory diseases such as sepsis-induced lung injury [10, 15, 25, 34, 40]. We primed canine granulocytes with 5 ng/ml rcGM-CSF for 15 min. These priming conditions have previously been used to prime human neutrophils with human recombinant GM-CSF and have been reported to be sufficient to enhance ROS production [16]. However, cellular reactivity to priming agents may be species-specific. Lipopolysaccharide (LPS), a well-known priming agent, upregulates CD11b in human, murine and equine granulocytes [33, 36, 41], but not in bovine granulocytes [29]. Therefore, species-specific differences in reactivity to GM-CSF may occur in the same manner as for LPS. Our results indicate that rcGM-CSF significantly upregulated CD11b expression, shortened PT and enhanced ROS production. These results confirm that rcGM-CSF can prime canine granulocytes in a similar manner as human granulocytes.

Some reports have suggested that Lido attenuates the priming of human neutrophils. Jinnouchi et al. reported that Lido inhibits LPS priming of human neutrophils for the oxidative burst. This inhibition was observed at concentrations above 1 mM (approximately 234.3 μg/ml) [23]. Ohsaka et al. reported that 5 mg/ml Lido (which exceeds the clinically relevant concentration of Lido) inhibits priming of human neutrophils by hrG-CSF for the oxidative burst [31]. However, the effects of Lido on rcGM-CSF-induced priming of canine granulocytes have not been reported previously.

We showed that the rcGM-CSF-induced enhancement of CD11b expression is attenuated by preincubation with 300 μg/ml Lido, but that lower concentrations of Lido had no effect. In addition, at a concentration of 300 μg/ml, Lido tends to inhibit the shortening of PT and the enhancement of ROS production by rcGM-CSF. These results are consistent with the data of Jinnouchi et al. [23] and suggest that 300 μg/ml Lido suppresses the priming of canine granulocytes by rcGM-CSF for CD11b expression and the oxidative burst. However, granulocytes can be primed by various agents other than GM-CSF, and each priming agent follows a specific route of signal transduction. Therefore, further studies on the effects of Lido on the priming of canine granulocytes by other priming agents are required.

In canine leukocyte adhesion deficiency (CLAD), CD11b is not expressed on the granulocyte surface; thus, life-saving antibiotic therapy against infectious diseases is required. Our data suggested that the magnitude of suppression observed in stationary canine granulocytes is moderate compared with that observed in CLAD [35]. To our knowledge, however, there have been no reports on Lido-induced infectious diseases. Thus, it is uncertain whether the suppression of canine granulocytes or rcGM-CSF priming by Lido is a direct trigger for infection. However, Lido is generally applied with other anesthetics (including general anesthetics, such as propofol), which are known to affect granulocyte function [21], and such agents may work synergistically to increase the risk of infectious diseases.

In conclusion, regardless of its efficacy as an analgesic, Lido has the potential to be a risk factor for infection depending on patient condition or other drugs used. The greatest risk that needs to be considered is the possibility of infection when performing regional blockade, such as brachial plexus block or epidural infusion. Furthermore, the body constitution differs vastly between dog breeds, and inter-breed differences in the activity of drug-metabolizing enzymes are known. These factors may lead to differences in Lido concentrations in blood or tissue in each breed. Thus, further study is needed with regard to the effects of Lido on canine granulocyte function in vivo and with regard to Lido concentrations in blood and tissue in relation to various analgesic methods.

REFERENCES


