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The Effect of Intravenous Lidocaine Infusion on Bronchoalveolar Lavage Cytology in Equine Recurrent Airway Obstruction

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Background: Lidocaine decreases neutrophilic inflammation in models of acute lung injury and decreases inflammation in asthmatic patients. Neutrophilic bronchiolitis develops in recurrent airway obstruction (RAO), but it remains unknown if lidocaine infusion decreases neutrophil migration into the airways.

Hypothesis: Lidocaine decreases neutrophilic inflammation as measured in BALF in RAO-affected horses.

Animals: Six RAO-susceptible horses in remission.

Methods: In a randomized cross-over design, horses received lactated Ringer's solution (LRS) IV or lidocaine hydrochloride IV with a minimum of 4 weeks at pasture between treatments. Treatments were delivered as continuous infusions beginning 4 hours before and for 68 hours during exposure to hay and straw challenge. Clinical score (CS, grade 0–8), maximal change in pleural pressure (ΔPpl_{max}), and bronchoalveolar lavage fluid (BALF) cytology were measured at baseline and the end of challenge (day 4). Plasma lidocaine concentrations were monitored daily.

Results: At baseline, there were no significant differences in variables between treatments. Plasma lidocaine concentration was consistently > 1100 ng/mL. After challenge, CS increased significantly [baseline: 2/8 (2–3), [median (interquartile range)]; day 4: 4/8 (4–5) $P = .0006$] as did ΔPpl_{max} [baseline: 3.6 (2.63–4.95) cmH_20; day 4: 9.62 (6.5–16) $P = .0036$], but there was no difference between treatments. Percentage of neutrophils was not different between treatments, but lidocaine infusion significantly increased BALF total cells [baseline: LRS $2.18 \pm 0.82 \times 10^5$ cells/mL (mean ± SD), lidocaine $1.6 \pm 0.3 \times 10^5$, day 4: LRS $2.0 \pm 0.88 \times 10^5$, lidocaine $4.4 \pm 2 \times 10^5$ ($P = .0045$)].

Conclusions and Clinical Importance: Lidocaine does not decrease neutrophilic inflammation in RAO.

Key words: BALF; Heaves; Lidocaine hydrochloride; RAO.

In addition to being a local anesthetic, lidocaine has potent anti-inflammatory properties, which are exerted on a variety of cell types by several mechanisms. In particular, lidocaine impairs neutrophil function by decreasing priming, respiratory burst, superoxide production, and production of cytokines. Lidocaine also decreases expression of adhesion molecules, which impairs neutrophil extravasation.[1–3] Prophylactic lidocaine therapy has shown promise in animal models of acute lung injury where it decreases pulmonary neutrophilic inflammation.[4] Asthmatic patients treated with nebulized lidocaine show improved clinical signs and lung function and decreased reliance on bronchodilator therapy. These improvements are attributed to both anti-inflammatory and neurally mediated mechanisms.[5,6]

Recurrent airway obstruction (RAO) is a hypersensitivity disease typified by extravasation of neutrophils into the airways and development of airway obstruction after exposure to elements present in hay. The nonseptic neutrophilic bronchiolitis that develops contributes to the inflammatory process by producing proinflammatory cytokines, proteolytic enzymes, eicosanoids, and reactive oxygen species (ROS).[7–10] Thus, a compound, such as lidocaine that decreases neutrophil extravasation, could represent a valuable agent to aid further investigation into the role of the neutrophil in RAO. Furthermore, although IV lidocaine would be an impractical therapy for RAO in the field, evidence of beneficial effects could prompt further investigation of nebulized lidocaine as a possible treatment. Therefore, we hypothesized that treatment with lidocaine decreases neutrophilic inflammation in RAO-susceptible horses during a natural challenge.

Abbreviations:

ΔPpl_{max}	maximal change in pleural pressure
BAL (F)	bronchoalveolar lavage (fluid)
LRS	lactated Ringer's solution
RAO	recurrent airway obstruction

Materials and Methods

Animals

Six horses of various breeds (5 mares, 1 gelding; 24 ± 4.6 [mean ± SD] years of age), weighing 473 ± 44 kg with a history of RAO were used for this study, which was approved by Michigan State University Institutional Animal Care and Use Committee. All horses had been donated to the Michigan State University's Pulmonary Laboratory RAO herd and had a history of increased respiratory effort when exposed to hay. Their clinical signs resolved when horses were placed at pasture. Diagnosis was confirmed by increased maximal change in pleural pressure (ΔPpl_{max} > 15 cm H_20) during hay and straw challenge and > 50% reduction in ΔPpl_{max} after IV injection of atropine sulfate[a] (0.02 mg/kg).[11] In the present study, all horses were kept on pasture and their diet was supplemented with complete pelleted feed except during the protocols.

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Clinical Score and Maximal Change in Pleural Pressure

Clinical score was assessed using a cumulative 8-point score as previously described.[12] Briefly, scores of 0–4/8 are consistent with horses in remission and scores of 5–8/8 are indicative of clinical RAO. Clinical score was agreed upon by 3 observers (MEW, CB, AB). Investigators were not blinded to the treatment.

The maximal change in pleural pressure was measured using an esophageal balloon.[13] The difference between the minimum and maximum pleural pressure (ΔPpl_{max}) during 20 breaths was calculated as an assessment of pulmonary function.

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed as previously described.[13] Lavage fluid was pooled, total cell number/mL was counted using a hemocytometer, and the percentage of each type of inflammatory cell was determined by counting 300 cells on a modified Wright Giemsa cytocentrifuged preparation. Estimates of neutrophil, macrophage, and lymphocyte cell numbers were calculated by multiplying the total cell number by the cell percentage.

Lidocaine Preparation and Measurement

Lidocaine hydrochloride[b] was added to LRS to produce a 6.6 mg/mL solution that was delivered by continuous rate infusion[c] at 0.08 mg/kg/min. LRS was delivered at 500 mL/h to approximately match the volume of lidocaine treatment. Continuous infusion was ensured by frequent monitoring of the fluid delivery set and continuous monitoring of the infusion pump audible alarm.[d]

Plasma concentrations of lidocaine and its metabolites monoethylglycinexylidide (MEGX) and glycinexylidide (GX) were measured in blood that was collected in sodium heparin tubes from the noncatheterized vein. Blood was centrifuged and plasma was stored at −80°C until analysis at a commercial laboratory.[e]

Experimental Protocol

In a randomized cross-over design, horses received lidocaine as either the 1st or 2nd treatment. A minimum of 4 weeks elapsed between 1st and 2nd treatments. Two to 3 horses received treatment simultaneously. While the horses were at pasture, clinical score and ΔPpl_{max} were measured, and at baseline (day 0), BAL was performed. Three days later, the horses were transported to a well-ventilated stall devoid of hay or straw and allowed to settle for 1–2 hours. Blood then was collected for measurement of lidocaine concentration (0 hours) and ΔPpl_{max} was measured. An IV catheter then was aseptically placed and secured in the jugular vein and treatment was initiated. Four hours later, a 2nd blood sample was collected for measurement of plasma lidocaine concentration, measurement of ΔPpl_{max} was repeated, and the horses then were relocated to a poorly ventilated stall where they were exposed to hay and straw (natural challenge) for 68 hours to induce pulmonary inflammation. Because plasma lidocaine concentrations can decrease rapidly after cessation of infusion, treatments were continued throughout relocation and the entire natural challenge period. During lidocaine administration, horses were observed daily for neurological signs because these are indicative of lidocaine toxicity. At the end of treatments (day 4), ΔPpl_{max} was measured and BAL performed on the contralateral lung to that used at pasture.

Data Analysis

Normality of the errors of each variable was assessed by visual inspection of the error histogram and probability plots and by testing normality using the univariate procedure in SAS.[f] Errors that were not normally distributed were log transformed (ΔPplmax, clinical score, number of macrophages and percentage of neutrophils) or Box-Cox transformed (number of neutrophils) using the TRANSREG[g] procedure. Normality of transformed data was assessed as described above. There was no effect of sequence of treatment; therefore, data were analyzed using a 3-factor ANOVA with the fixed effects of treatment and time and the random effect of horse (SAS PROC MIXED). Significance was set at ($P < .05$). Lidocaine and metabolite MEGX and GX concentrations over time were analyzed using 1-way repeated measures ANOVA.

Results

Plasma Lidocaine Concentration

Plasma lidocaine and its metabolites were below limits of detection before lidocaine infusion (Fig 1) and during LRS treatment (data not shown). Plasma lidocaine concentration reached 1,945 ± 368 ng/mL (mean ± SD) after 4 hours of infusion and remained stable thereafter. Only 1 horse developed neurologic signs. This horse became ataxic after 24 hours of lidocaine infusion, but ataxia resolved when lidocaine transfusion was stopped for 10 minutes and the initial infusion rate was decreased. Although lidocaine infusion does decrease fecal output in horses without gastrointestinal disease,[14] fecal output was not specifically quantified in the present investigation. However, colic was not observed in any horse during the study.

Mean concentrations of MEGX increased during the first 24 hours of lidocaine administration and then reached a plateau. The metabolite GX increased rapidly during the first 24 hours and continued to increase such that concentrations at 72 hours were significantly greater than those at 24 hours ($P = .005$). There were no differences in plasma lidocaine concentrations among horses, but there was a significant effect of horse on serum concentrations of GX and MEGX.

Clinical Score and Pulmonary Function

At no time during the protocol was there a significant difference in clinical score or ΔPpl_{max} between LRS and lidocaine treatments. However, both significantly increased by the end of challenge [Clinical score, baseline: 2/8 (2–3), [median (interquartile range)]; day 4: 4/8 (4–5) $P = .0006$] and ΔPpl_{max} [baseline 3.6: (2.63–4.95) cmH_20; day 4: 9.625 (6.5–16) $P = .0036$] (Fig. 2).

Bronchoalveolar Lavage Fluid Cytology

There was no significant difference in the volume of BAL fluid retrieved at pasture and postchallenge, and neither LRS nor lidocaine treatments had any effect on the percentage of infused volume retrieved [LRS, baseline: 36.2 ± 5.34%; (mean ± SD); day 4:

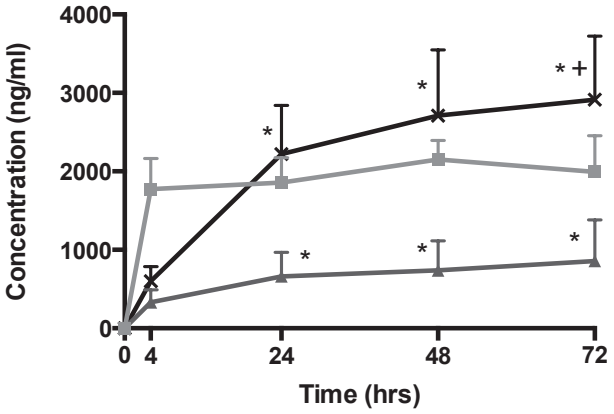

Fig 1. Serum concentrations (mean + SD) of lidocaine (square), MEGX (triangle) and GX (cross) over the duration of lidocaine infusion. *$P < .05$ when compared with 4 hours within compound. †$P < .05$ when compared with 24 hours within compound.

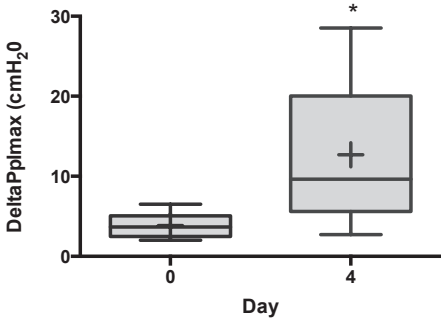

Fig 2. Maximal change in pleural pressure (ΔPplmax) in RAO-affected horses at baseline (day 0) and after 3 days exposure to natural challenge (day 4). Data are presented as box plots; open diamond indicates mean. There was no effect of treatment with either LRS or lidocaine and data are combined. After natural challenge, there was a significant increase in ΔPplmax (*$P = .0036$).

23 ± 12.3; lidocaine, baseline: 33.2 ± 15.9; day 4: 30.9 ± 15.8].

Total cell number, percentages and absolute numbers of neutrophils, macrophages, and lymphocytes in BALF were not different before treatment and there was no effect of the sequence of treatments. The total cell count remained stable in LRS treatment during natural challenge (Fig 3). However, by day 4 of lidocaine treatment, the total cell number was significantly increased ($P = .005$) and was significantly greater than in LRS treatment ($P = .009$). The increased total cell count was principally explained by a substantially increased neutrophil cell number during lidocaine treatment (day 0, 0.03 (0.02–0.19) [$\times 10^5$ cells/mL; median, (interquartile range)]; day 4, 1.79, (0.9–4.07) $P = .005$) compared with a lesser increase in neutrophil cell numbers during LRS treatment (day 0, 0.11 (0.07– 0.2); day 4, 0.55 (0.46–1.05) $P = .11$). Despite the magnitude of neutrophil cell numbers being higher in lidocaine treatment compared with LRS treatment by day 4, they were not significantly different from each other.

Macrophage cell number significantly decreased during LRS treatment (day 0, 0.57 (0.48–0.83) [$\times 10^5$ cells/mL; median, (interquartile range)]; day 4, 0.26, (0.22–0.33) $P = .02$), whereas during lidocaine treatment macrophage number increased, but not significantly (day 0, 0.34, (0.26–0.63), day 4, 0.67 (0.49–0.83), $P = .052$) and, by day 4, macrophage cell number was significantly higher in the lidocaine treatment compared with the LRS treatment ($P = .006$). There was no significant effect of natural challenge or treatment on lymphocyte numbers (LRS, day 0, 1.33 ± 0.68 (mean ± SD), day 4, 0.85 ± 0.46; lidocaine, day 0, 1.06 ± 0.4, day 4, 1.02 ± 0.7).

Characteristic of RAO, the percentage of neutrophils was significant increased by day 4 during natural challenge ($P = .007$) (Fig 4). Although there was a significant day by treatment interaction with a significant increase within lidocaine treatment ($P = .014$), there was no significant difference between treatments by day 4. Although the percentage of lymphocytes decreased significantly by day 4 ($P = .02$), examinations of interactions indicated that this was only significant during lidocaine treatment ($P = .02$). Similarly, by day 4, the percentage of macrophages decreased significantly only during LRS treatment ($P = .02$).

Discussion

The aim of this randomized, cross-over study was to determine if prophylactic treatment with lidocaine decreases the neutrophilic inflammation associated with development of RAO. This study demonstrated that

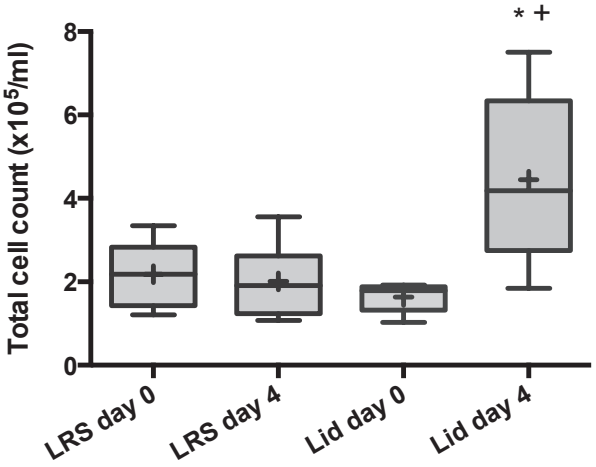

Fig 3. Box plots of BALF total cell count for lidocaine and LRS treatments at baseline (0) and on day 4 of challenge (4) (open diamond indicates mean). *$P = .005$ when compared to day 0 within treatment. †$P = .009$ when compared to LRS at the same time point.

lidocaine did not alter the composition of the BALF cytology, but, in contrast, significantly increased total numbers of inflammatory cells in BALF.

In other species, lidocaine therapy decreases extravasation of neutrophils into pulmonary tissue during a number of challenges.[4,15,16] This may occur by means of direct inhibition of neutrophil and endothelial adhesion molecules[2] or may be a consequence of the combined anti-inflammatory spectrum, such as attenuated NFκB activation, and decreased production of reactive oxygen species and acute phase cytokines.[16–18] The chronic inflammation associated with RAO entails stimulation of TLR4 and NFκB signaling, and release of ROS and acute phase cytokines.[19–22] On the basis of lidocaine's broad anti-inflammatory effects, it was hypothesized that a reduction in severity of neutrophilic inflammation and possibly the severity of respiratory dysfunction would be observed in RAO-affected horses.

However, consistent with our findings, recent investigations indicate an absence of anti-inflammatory effects of lidocaine on equine neutrophils. Lidocaine infusion does not decrease neutrophilic migration into the peritoneum in experimental endotoxemia[23] or into the laminar interstitium in black walnut extract-induced laminitis.[24] In vivo studies similarly indicate that lidocaine does not decrease equine neutrophil activation, expression of adhesion molecules, or migration. In fact, IV lidocaine actually may promote inflammation by upregulation of equine endothelial adhesion molecules.[24] Furthermore, supraphysiologic concentrations of lidocaine promote equine neutrophil migration *in vitro,* although this occurred at a concentration more than 500 times higher than the plasma concentrations detected in the present study.[25] These results indicate that not all mammalian neutrophils behave similarly in response to lidocaine.

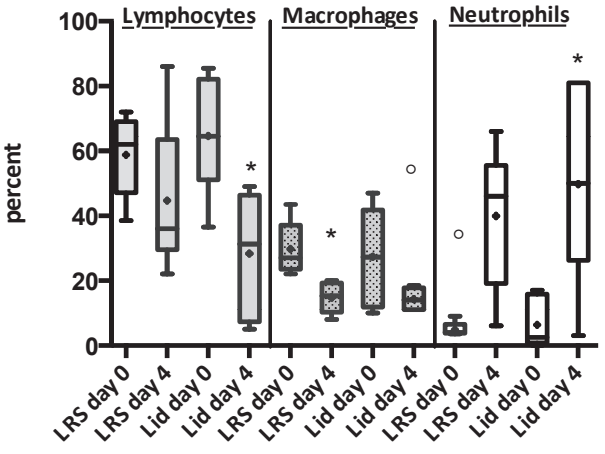

Fig 4. Box plots of BALF differential cell count; lymphocytes, macrophages, and neutrophils are expressed as a percentage of total cells for lidocaine and LRS treatments at baseline (day 0) and on day 4 of challenge. (° indicates outliers, open diamond indicates mean.) $*P < .05$ when compared to time 0 within treatment.

Although a greater number of total inflammatory cells was detected in BALF after lidocaine treatment, we cannot speculate on the functionality of these cells. It remains unknown if they contributed to the inflammatory milieu in a similar manner to the LRS-treated cells. Although the data from lidocaine-treated equine neutrophils suggests lidocaine does not impair function, the effects on equine macrophages and lymphocytes have not yet been investigated. Despite the increased total cell count after lidocaine treatment, the differential cell count remained similar compared to horses after LRS treatment. This result could be a consequence of impaired pulmonary clearance of inflammatory cells. Intravenous lidocaine has an antitussive effect in nonanesthetized people[26] and topical lidocaine applied to the central airways prevents coughing in response to bronchoscopy in horses.[27] Furthermore, topical lidocaine decreases ciliary beating frequency and mucus transport velocity in larger airways, although it remains unclear if this occurs with IV delivery.[28] Coughing is a prominent clinical sign during active RAO[29] and it is possible that both impaired mucociliary clearance and inhibited cough reflex delayed removal of inflammatory cells from the lower airways resulting in the increased cell numbers.

During RAO exacerbation, the large influx of neutrophils into the airways (accompanied by lesser alterations in lymphocyte and macrophage cell numbers) typically accounts for the increased total cell count commonly observed in BALF. In this study, LRS-treated horses had increased neutrophil percentages, but no increases in total cell count. This observation may be explained by the fact that horses were in the acute, developmental stage of RAO exacerbation (based on clinical score and pulmonary function). Although it remains somewhat unusual for the neutrophil number and percentage to increase in RAO without increasing the total cell count, this may occur in RAO-affected horses during the acute phase of RAO[30] and in normal horses exposed to stable environment.[31] Presumably, with early or low grade inflammation, movement of lymphocytes or macrophages out of the airway lumen balances the moderate numbers of neutrophils that enter the lumen.

Total cell count of BALF can be influenced by the dilution of the pulmonary epithelial lining fluid and the volume of the aliquot of sample analyzed. Measurement of dilutional markers, such as urea and albumin,[32] can be used to overcome this variability. In this study, dilutional markers were not measured, which is a limitation; however, each BAL was performed by the same investigator (CB) using a standardized technique,[13] and all retrieved fluid was pooled together before analysis. Furthermore, there was no difference in percentage of lavage fluid retrieved between either LRS or lidocaine treatments. The low percentage of retrieved fluid (approximately 30% for all lavages) is typical when sampling RAO-affected horses.[33]

The purpose of this study was to assess the effect of lidocaine on the development of neutrophilic inflammation in RAO and because this occurs early in the course of the disease, we selected a relatively short duration of natural challenge (68 hours). Although this time period was sufficient to elicit neutrophilic inflammation typical of RAO, this short exposure limited the severity of disease as evidenced by the relatively mild deterioration of pulmonary function (clinical score and ΔPpl_{max}). Nebulized lidocaine therapy in asthmatic patients is associated with improved pulmonary function. In this study, lidocaine treatment had no effect on clinical score or pulmonary function, but this is not unexpected because the model did not induce severe pulmonary dysfunction, and clinical score and ΔPpl_{max} are relatively insensitive at detecting small changes in airway dysfunction.

In this study, lidocaine was delivered at a dosage of 0.08 mg/mL/min. In practice, lidocaine infusion is commonly used as a treatment for equine ileus and frequently is delivered at a lower dosage of 0.05 mg/kg/min and achieves a target steady state of approximately 980 ng/mL.[34] However, at the latter dosage, lidocaine does not exert its beneficial effect as a consequence of impaired neutrophil influx into intestinal mucosa, although other anti-inflammatory mechanisms may be operative.[35] Because in vitro studies of bovine and canine peripheral blood demonstrate decreased adhesion molecule expression in a dose-dependent manner[3,36] and as the threshold for serum lidocaine toxicity is double that of 980 ng/mL,[37] we elected to deliver a higher dosage to potentially maximize our ability to detect a significant lidocaine effect on neutrophilic inflammation. However, in rabbit studies of endotoxin-induced acute lung injury,[38] delivery of IV lidocaine at 0.03 mg/kg/min (plasma lidocaine concentrations not provided) significantly decreases neutrophilic infiltration into pulmonary parenchyma. Therefore, it can be assumed that our findings were not attributable to inadequate lidocaine concentrations. In accordance with a previous study, the metabolite MEGX reached stable concentrations, whereas GX continued to accumulate over the duration of the infusion.[39] The effects of these metabolites on neutrophil and pulmonary function remain unknown.

The horses used in this study were older, but there was no evidence of either cardiac disease or hepatic dysfunction, which can predispose to lidocaine toxicity. Although advancing age is associated with an exaggerated inflammatory response,[40] there is no evidence that RAO severity increases with age, and thus it is unlikely that a younger cohort of RAO-affected horses would respond differently to lidocaine treatment.

In summary, this study indicates that lidocaine does not decrease the severity of neutrophilic inflammation as measured by BALF in RAO-affected horses, but is associated with an increased BALF total cell number. This information adds to reports supporting that lidocaine does not alter neutrophil migration in horses. The underlying mechanisms of the increased cell count and potential consequences await additional investigation.

Footnotes

[a] Atropine sulfate 0.4mg/mL, Baxter Healthcare Corporation Deerfield, IL
[b] Lidocaine Hydrochloride 2%, Vedco Inc, St Joseph, MO
[c] Baxter Flo-Gard® 6201, Volumetric Infusion Pump
[d] Sony NTM910 Baby Call Nursery Monitor
[e] Department of Comparative Medicine, University of Tennessee Veterinary Teaching Hospital, Knoxville, TN
[f] SAS Institute Inc. 2004. SAS® 9.1
[g] SAS PROC TRANSREG

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Conflict of Interest: Authors disclose no conflict of interest.

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