Local Anesthetics Time-Dependently Inhibit *Staphylococcus aureus* Phagocytosis, Oxidative Burst and CD11b Expression by Human Neutrophils

Annette Ploppa, M.D., Ralph-Thomas Kiefer, M.D., Wolfgang A. Krueger, M.D., Klaus E. Unertl, M.D., and Marcel E. Durieux, M.D., Ph.D.

Background and Objectives: Local anesthetics have been shown to modulate neutrophil functions in a time-dependent manner, which might help to prevent inflammatory injury to the organism. However, if host defense mechanisms are affected similarly, the ability to eliminate bacteria might be reduced. We hypothesized that local anesthetics have time-dependent effects on phagocytosis of *S. aureus*, oxidative burst, and CD11b expression by human neutrophils. To test this hypothesis, we reanalyzed data from a previous study.

Methods: Blood samples from 11 healthy volunteers were incubated with lidocaine (1,846 μmol/L), bupivacaine (770 μmol/L) or ropivacaine (801 μmol/L) for 30 minutes. Thereafter, bacteria were added, either fluorescently labeled for determination of phagocytosis, or unstained for determination of oxidative burst and CD11b expression. After an additional incubation for 0, 10, 30, or 60 minutes, phagocytosis was stopped and neutrophils were stained with monoclonal antibodies for flow cytometric analysis. Data were analyzed by analysis of variance for repeated measurements.

Results: Lidocaine and bupivacaine inhibited neutrophil functions in a time-dependent manner ($P < .05$). Prolonged local anesthetic exposure reduced the fraction of ingesting neutrophils by 20% ± 12% (mean ± SD) and 7% ± 7%, bacterial uptake by 19% ± 16% and 14% ± 12%, oxidative burst by 29% ± 23% and 28% ± 25%, and CD11b expression by 66% ± 24% and 25% ± 21% for lidocaine and bupivacaine, respectively. Ropivacaine exerted a time-dependent effect on CD11b expression only (24% ± 34%; $P < .05$).

Conclusions: Our results indicate that in a whole blood model, time-dependent effects of local anesthetics affect key neutrophil functions necessary for bacterial elimination. However, these effects only occur at concentrations that are unlikely to be routinely attained in the clinical setting, and concern about interfering with the host defense is likely unwarranted. *Reg Anesth Pain Med 2008;33:297-303.*

Key Words: Time dependence, Local anesthetics, Ropivacaine, Bupivacaine, Lidocaine, Host defense, Phagocytosis, Oxidative burst, CD11b.

Systemic local anesthetic (LA) administration accelerates recovery of bowel function and reduces pain after surgery. Significant reductions in hospital length of stay were observed when lidocaine was administered intravenously during prostatectomy or laparoscopic colectomy.[1,2] The effect size of reduction in postoperative ileus duration is similar to that obtained using epidural local anesthetic administration, suggesting that the benefit of epidural therapy may result in part from the systemic local anesthetic levels attained using this technique.[3,4]

A modulatory effect of LA on the inflammatory response to surgery may be partially responsible for these benefits, as suggested by findings that LAs can affect neutrophil signaling.[5-7] We previously reported that exposure of isolated human neutrophils to LA for several hours (in concentrations as attained in blood during epidural or intravenous in-

From the Departments of Anesthesiology and Intensive Care Medicine (A.P., R.-T.K., W.A.K. Professor, K.E.U. Professor and Chair.), Eberhard-Karls University, Tübingen, Germany; and the Department of Anesthesiology (A.P., M.E.D. Professor), University of Virginia, Charlottesville, VA.

Accepted for publication May 22, 2007.

This study was financed by institutional scientific budgets and a Carl Koller award from the American Society for Regional Anesthesia (to M.E.D.).

Reprint requests: Annette Ploppa, M.D., Department of Anesthesiology and Intensive Care Medicine, Eberhard-Karls University Tübingen, Hoppe-Seyler-Str. 3, 72 076 Tübingen, Germany. E-mail: annette.ploppa@uni-tuebingen.de

1098-7339/08/3304-0001$34.00/0
doi:10.1016/j.rapm.2007.05.012

fusion) inhibits priming of oxidative burst by more than 50%. Importantly, this effect is profoundly time-dependent: the sensitivity of neutrophils to LA increases with the duration of LA exposure. This may explain why only prolonged administration of LA has notable benefits in the clinical setting.

However, modulating inflammatory responses might also affect the host defense against bacterial invasion, which is mediated by several important neutrophil functions such as endothelial adherence, phagocytosis, and oxidative burst. These critical functions are known to be inhibited by high concentrations of local anesthetics, but it is not known if local anesthetic sensitivity increases in a time-dependent manner.[8]

One year prior to reporting the time-dependent inhibition of oxidative burst in isolated neutrophils, we published a study of local anesthetic effects on several measures of host defense.[8] This study was performed in human whole blood using live *S. aureus* as stimulus, and neutrophil oxidative burst, phagocytosis activity, and CD11b expression were investigated. We subsequently realized that this study design allowed us to investigate the time-dependent effects of local anesthetics on host defense functions, while correcting several weaknesses of the isolated cell model. The use of a whole blood model instead of isolated neutrophils and the application of a clinically relevant stimulus, such as exposure to bacteria, would provide a more realistic setting.

Although the study in whole blood was not designed to investigate time dependence, we had exposed neutrophils to local anesthetics for various durations of time. We realized that we might be able to retrospectively study: (1) whether time-dependent inhibition of oxidative burst occurred in the more realistic settings of the whole blood model; and (2) whether time-dependent actions on phagocytosis and CD11b expression could be observed. We therefore reanalyzed the original data of the whole blood study, including some data not presented in the earlier manuscript. We hypothesized that, similar to their effect on respiratory burst, local anesthetics will affect phagocytosis and CD11b expression in a time-dependent manner.

Methods

The study was performed at the Eberhard-Karls University, Tübingen, Germany, after obtaining Institutional Review Board approval and written informed consent from participants. Detailed methods were described in our previous paper.[8] For each assay, blood samples from 11 volunteers were incubated with lidocaine (9.2 (2.5 μg/mL), 92.2 and 1846 μM), bupivacaine (6.1 (2 μg/ml), 61 and 770 μM), ropivacaine (6.4 (2 μg/ml), 64 and 801 μM) or equal volumes of 0.9% NaCl for 30 minutes (all local anesthetics from Astra; Wedel, Germany). For comparative purposes, peak plasma levels for lidocaine range from 20 to 36 μmol/L after peripheral nerve block and from 15 to 20 μmol/L after epidural anesthesia. Samples were then exposed to an equal volume of RPMI (RPMI 1640, without calcium and magnesium, Sigma, Deisenhofen, Germany) with 10% autologous serum, containing live *S. aureus* ATCC 25923 in a 5:1 ratio of bacteria to neutrophils for 0, 10, 30, or 60 minutes at 37°C. For quantification of phagocytosis by flow cytometry, bacteria were stained with Calcein-AM (Molecular Probes, Eugene, OR). For quantification of oxidative burst with dihydroethidium (Sigma, Deisenhofen, Germany) and CD11b expression, unstained bacteria were used in the same concentration as for the phagocytosis assay. Phagocytosis was stopped using N-ethylmaleimide (NEM; Sigma, Deisenhofen, Germany) and thereafter by keeping the samples cooled with ice. After staining with monoclonal antibodies (CD13, DAKO, Glostrup, Denmark; and CD11b Caltag Laboratories, San Francisco, CA) and lysis of erythrocytes, samples were evaluated by flow cytometry (FACS SORT; software Cell Quest, Becton Dickinson, Heidelberg, Germany). Because of the different progress of neutrophil functions, phagocytosis was allowed to proceed for 60 minutes whereas respiratory burst and CD11b expression assays were stopped after 30 minutes.

Simultaneous addition of both *S. aureus* and the phagocytosis inhibitor (NEM) was defined as time-point 0 and used as baseline for the calculation of time dependency (these data were not presented in our previous report).

Ten thousand neutrophils were analyzed in each sample. Phagocytosis was assessed in 2 ways: first, as the fraction of neutrophils actively ingesting *S. aureus,* and second, as the fluorescence intensity of ingested bacteria (FIB), a semiquantitative measure of the bacterial load of each neutrophil. For each time point and experimental condition, we subtracted results obtained in the presence of local anesthetics from those in their absence. Statistical analysis was performed using SigmaPlot 10/SigmaStat 3.5 (Systat Software, Point Richmond, CA). Time dependence of the data was analyzed by 1-way analysis of variance for repeated measures (ANOVA-RM), or, if data were not normally distributed, by ANOVA-RM on ranks. If significant ($P < .05$), corrected multiple comparisons versus baseline at 0 minutes was performed using the Holm-Sidak method (or Dunn's test if data were not normally distributed).

In order to compare the effect size of the time-dependent inhibition induced by the three LA, we used data obtained at 30 minutes. We calculated the