Ropivacaine Inhibits Leukocyte Rolling, Adhesion and CD11b/CD18 Expression

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ABSTRACT

Ropivacaine, a new local anesthetic, is currently being investigated for the treatment of ulcerative colitis, with promising results so far. The aim of this study was to examine anti-inflammatory properties of ropivacaine with regard to its effects on vascular permeability and inflammatory leukocyte behavior in vivo. The effects on leukocyte rolling, firm adhesion and vascular permeability were examined in the hamster cheek pouch microvasculature by intravital microscopy, and the effects on leukocyte adhesion molecules were examined in vitro by means of flow cytometry. Large venules, leukocyte adhesion induced by topical leukotriene B4 (LTB4) was almost completely inhibited during the combined application of ropivacaine and LTB4. The spontaneous rolling leukocyte flux was reduced by 72%, the rolling leukocyte fraction by 47% and the total leukocyte flux, which reflects blood flow, by 47%. In postcapillary venules, ropivacaine abolished rolling and LTB4-induced firm adhesion of leukocytes. LTB4 challenge also resulted in increased plasma exudation that was almost completely inhibited by ropivacaine. Moreover, ropivacaine inhibited the tumor necrosis factor α-induced up-regulation of CD11b/CD18 and L-selectin shedding by human leukocytes in vitro. Our results suggest that ropivacaine exerts anti-inflammatory activity, and this appears to be mediated to a significant extent by inhibition of both leukocyte rolling and adhesion.

Local treatment of UC with lidocaine has been shown to result in good symptomatic relief and restored mucosal integrity (Bjöck et al., 1993). Ropivacaine, a new local anesthetic currently under investigation for the treatment of UC, appears to improve inflammatory endoscopic scores and to decrease clinical symptoms after only 2 weeks of treatment (Arlander et al., 1996). Furthermore, ropivacaine has protective effects in a rat colitis model (T. Martinsson, unpublished observation).

In addition to reversible block of nerve impulse propagation, local anesthetics are known to affect a variety of other cell functions (Hammer et al., 1985; Moudgil et al., 1977; Dickstein et al., 1985). Many of these effects are related to leukocyte function; local anesthetics have been shown to inhibit leukocyte phagocytic activity (Cullen and Haschke, 1974), superoxide production (Peck et al., 1985; Irita et al., 1986) and adhesion (Giddon and Lindhe, 1972; Rabinovitch and DeStefano, 1974; MacGregor et al., 1980). Lidocaine also reduces leukocyte adherence in injured venules in vivo (Stewart et al., 1974), counteracts the endothelial damage induced by sticking leukocytes (Stewart et al., 1974) and inhibits granulocyte recruitment to sites of inflammation (MacGregor et al., 1980). Ropivacaine itself has recently been shown to inhibit release of LTB4 and 5-hydroxyicosatetraenoic acid from leukocytes (Martinsson et al., 1997).

The migration of leukocytes into tissues is a crucial event in the inflammatory response. Leukocyte emigration is normally responsible for the successful host response to tissue injury and infection, but it is also potentially harmful and contributes to the pathology of different inflammatory diseases such as UC (Babbs, 1992). Accordingly, it has been shown that suppression of neutrophil function may reduce tissue damage in inflammatory diseases (Fujita et al., 1994), including UC (Palmen et al., 1995). The accumulation of leukocytes in inflamed tissue and the excessive filtration of fluid and proteins that accompanies an inflammatory response are largely confined to small venules in the microcirculation (Granger and Kubes, 1994). Leukocyte extravasation is initiated by interactions between circulating leukocytes and activated endothelial cells lining the venules of inflamed tissue. Rolling of leukocytes along the

ABBREVIATIONS: FITC, fluorescein isothiocyanate; fMLP, formyl-methionyl-leucyl-phenylalanine; LTB4, leukotriene B4; MFI, mean fluorescence intensity; PBS, phosphate-buffered saline; PE, phycoerythrin; TNF-α, tumor necrosis factor α; UC, ulcerative colitis.
venular wall is the earliest visible interaction. This is a reversible event that can be followed by either the release of the leukocytes back into the bloodstream or, upon chemotactic stimulation, by arrest and firm adhesion to the endothelium and subsequent diapedesis (Granger and Kubes, 1994). Recent studies have revealed that the leukocyte-endothelial interactions are mediated by different classes of cell surface adhesion molecules. These include the selectins, the integrins and members of the immunoglobulin superfamily (Springer, 1994). The selectins (E-, L- and P-selectin) are required for leukocyte rolling along the vessel wall (Ley and Pedder, 1998). L-selectin is constitutively expressed on leukocytes, whereas E- and P-selectin are inducible endothelial molecules (Springer, 1994). CD11b/CD18, a member of the integrin family, appears to be important for the firm adhesion of leukocytes to the endothelium (Arfors et al., 1987). This receptor is constitutively expressed on the surface of nonactivated leukocytes, and the surface expression of CD11b/CD18 may be further increased by mobilization from nonactivated leukocytes, and the surface expression of this receptor is constitutively expressed on the surface of activated leukocytes, whereas E- and P-selectin are inducible endothelial molecules (Springer, 1994). CD11b/CD18, a member of the integrin family, appears to be important for the firm adhesion of leukocytes to the endothelium (Arfors et al., 1987). This receptor is constitutively expressed on the surface of nonactivated leukocytes, and the surface expression of CD11b/CD18 may be further increased by mobilization from intracellular pools upon stimulation with various inflammatory stimuli (Todd et al., 1984).

Two important questions arise from the previous observations about the effects of local anesthetics on leukocyte activation and the promising results of ropivacaine in the treatment of UC. First, can ropivacaine inhibit leukocyte adhesion in vivo and/or the increased vascular permeability associated with inflammation? Second, can ropivacaine affect the expression of adhesion molecules on the surface of leukocytes? In the present study, we examined the effects of ropivacaine on increased vascular permeability and inflammatory leukocyte behavior in the hamster cheek pouch microvasculature by using intravital microscopy, and we examined its effects on the expression of adhesion molecules on human leukocytes in vitro by using flow cytometry.

Materials and Methods

Intravital Microscopy

Male golden hamsters (90–120 g, Harlan-CPB, Austerlitz, Netherlands) were used. The cheek pouch of sodium pentobarbital-anaesthetized animals was prepared for intravital microscopy according to Duling (1973) with modifications by Svensjö (1978) and Erlansson et al. (1989). The exposed cheek pouch was superfused with a bicarbonate-buffered salt solution (35°C) that was continuously equilibrated with 5% CO₂ in N₂ to maintain low oxygen tension (~4 kPa) and pH 7.35. A catheter in the left femoral vein was used for infusion of FITC-labeled dextran (MW 150,000, Bioflor HB, Uppsala, Sweden) or rhodamine (Sigma, St. Louis, MO). After positioning of the cheek pouch under the microscope (Axioskope, Zeiss), a 30-min equilibration period preceded the experiments. Rhodamine-labeled leukocytes in venules were visualized with fluorescent light epi-illumination using a ×25 water immersion lens (NA 0.60). The microscopic images were televised (Sony Trinitron, Sony CCD camera) and recorded with a Sony U-matic Video Tape Recorder for subsequent off-line analysis. In another set of experiments, nonlabeled leukocytes in postcapillary venules were studied with ordinary light transillumination using a ×40 water immersion lens (NA 0.90).

Vascular permeability. FITC-labeled dextran was injected i.v. (25 mg/100 g b.w.) as a macromolecular tracer. The increase in microvascular permeability for large molecules was quantified by counting fluorescent leakage sites at postcapillary venules (Erlansson et al., 1989). The number of leaks per square centimeter of cheek pouch area was counted before and during 30 min after topical application of LTB₄ (10 nM; Sigma; repeated four times with 45-min intervals). Ropivacaine (Naropin, Astra, Södertälje, Sweden) was applied to the superfusate for 15 min, starting 10 min before the second (10 μM ropivacaine) and the fourth (100 μM ropivacaine) LTB₄ applications. The peak number of leakage sites, which consistently occurred at 5 min after the start of LTB₄ application, was used for statistical calculations.

Leukocyte behavior. The cheek pouches were subjected to four repeated local applications of 10 nM LTB₄ for 5 min with 45- to 60-min intervals. Before the second and fourth LTB₄ applications, 100 μM ropivacaine was applied locally for 15 min starting 10 min before LTB₄; this resulted in two experimental series termed first and second. Venular segments with a diameter of 40 μm and a length of 150 μm were selected for observation of free-flowing, rolling and adherent leukocytes. All systemic leukocytes were labeled in vivo by an i.v. injection of rhodamine (2 μg) immediately before observations. The weak red fluorescence of rhodamine-labeled leukocytes was amplified using a Hamamatsu Image Intensifier and recorded for subsequent off-line analysis. Values for free-flowing, rolling and firmly adherent leukocytes were obtained immediately before LTB₄ application and during and after ropivacaine and/or LTB₄ application. Free-flowing leukocytes (with the same velocity as erythrocytes) were determined by counting the number of leukocytes passing a line perpendicular to the vessel per minute. Rolling leukocytes were defined as leukocytes that were in contact with the venular endothelium and had a velocity lower than that of free-flowing leukocytes, and the rolling leukocyte flux (cells per minute) was determined as described for free-flowing leukocytes. The leukocyte rolling fraction was determined at indicated time-points by dividing the rolling leukocyte flux by the total leukocyte flux (flux of rolling leukocytes plus that of free-flowing leukocytes). Cells were considered to be adherent if they remained stationary for more than 30 sec. Adherent cells were expressed as number of leukocytes (10,000 μm² inner surface of the vessel. In a second set of experiments, postcapillary venules with a diameter of 10 μm and a length of 100 μm were selected for observation of leukocytes with ordinary light transillumination. In these experiments, rolling leukocyte flux and adherent leukocytes were counted.

Vessel diameters. The diameters of venules and arterioles were measured off-line using an image-sensing monitor (IPM, LaMesa, CA).

Expression of Cell Surface Adhesion Molecules

Preparation of leukocytes. EDTA blood from healthy human donors (n = 19) was hemolyzed by dilution (1:20) in 4°C isotonic NH₄Cl-EDTA lysing solution (154 mM NH₄Cl, 10 mM KCl, 0.1 mM EDTA, pH 7.2). After incubation for 5 min at 15°C, the leukocyte pellets were centrifuged (300 × g, 4°C) for 5 min. The leukocyte pellets were washed once in 4°C 0.15 M PBS supplemented with 0.1 M EDTA and 0.02% sodium azide (PBS-EDTA). This cell preparation procedure minimizes spontaneous leukocyte activation (Lundahl et al., 1991).

Leukocyte activation. The basic medium used during leukocyte activation was RPMI 1640 (Gibco Ltd., Paisley, U.K.) containing 0.01 M HEPES and 5% fetal calf serum (Gibco Ltd.). TNF-α (R&D Systems, Abingdon, U.K.) was diluted to a final concentration of 10⁻⁹ g/ml. Ropivacaine and lidocaine (Xylocaine, Astra) were diluted in medium to make serial dilutions ranging from 10⁻⁵ M to 10⁻ⁱ M. The leukocytes were incubated with or without TNF-α for 15 min (L-selectin) or 30 min (CD11b/CD18), both at 37°C. The cells were treated with varying concentrations of ropivacaine, lidocaine or an equal volume of medium, added together with TNF-α. The activation was stopped by addition of cold PBS-EDTA, and the leukocytes were washed once, resuspended in 100 μl PBS-EDTA and kept on ice. As a control of spontaneous cell activation at 37°C, leukocytes were also incubated at 4°C without TNF-α and local anesthetics. The viability of the cells before and after incubation with ropivacaine or lidocaine was >95%, as determined by the trypan blue exclusion test.
Flow cytometric analysis of adhesion molecule expression. The expression of CD11b and L-selectin on granulocytes and monocytes was analyzed by adding 5 μl of PE-conjugated monoclonal anti-CD11b (DAKO A/S, Glostrup, Denmark) or 10 μl FITC-conjugated anti-L-selectin antibody (Becton Dickinson, Mountain View, CA) to the leukocytes prepared and treated as described above. The suspensions were incubated on ice for 30 min, washed in cold PBS-EDTA and resuspended in 0.5 ml of cold PBS-EDTA before analysis. Appropriate concentrations of iso- and subtype-matched control antibodies were used to define the cutoff for positive fluorescence. Positive fluorescence was the 99th percentile of the distribution of the cells labeled with the respective control antibody (PE-conjugated IgG2a and FITC-conjugated IgG2a for CD11b and L-selectin, respectively).

Finally, the cells were analyzed in an EPICS Profile II (Coulter Inc., Hialeah, FL) flow cytometer. Granulocytes and monocytes are represented by well-separated clusters based on light-scattering properties. Discriminating frames were placed around the granulocyte and monocyte fields. The instrument gives the actual number of cells in venules (ø 10 μm), superimposed by the leukocytes at different time-points, together with the rolling leukocyte flux. The base-line venular leukocyte adhesion was 10.2 ± 3.6 cells/10,000 μm². Superfusion with LTB₄ for 5 min significantly increased the number of adherent cells by 78%. This increase was reversible and returned to base line after termination of LTB₄ application. Addition of ropivacaine to the superfusion solution significantly reduced the leukocyte adhesion response to LTB₄ to a value comparable to the base-line value (fig. 2; table 1). Ropivacaine showed a tendency to reduce spontaneous adhesion, but this effect was not significant. The results could be repeated in the second series of experiments in the same preparation. In this second series, LTB₄ increased leukocyte adhesion by 115% (compared with 78% in the first LTB₄ application), and ropivacaine inhibited the induced adhesion completely (data not shown).

In postcapillary venules (ø 10 μm), LTB₄ increased endothelial-leukocyte adhesion almost 6-fold, and this increase was significantly inhibited by ropivacaine (table 1). There was a trend toward reduction by ropivacaine of the spontaneous leukocyte adhesion, but, as in the larger venules, the trend was not significant.

Leukocyte rolling. The base-line rolling leukocyte flux in venules was 26.6 ± 6.9 cells/min (table 1). As a result of the increased adhesion induced by LTB₄, rolling decreased markedly (94%) during the 5-min LTB₄ application (fig. 2). Ropivacaine reduced the spontaneous rolling flux by 72% without causing increased adhesion (fig. 2; table 1). During the combined application of ropivacaine and LTB₄, the rolling leukocyte flux was further reduced (76%) despite the inhibition of LTB₄-induced adhesion during this time. The leukocyte rolling fraction was calculated, and during base-line conditions, the fraction of rolling cells was 41% ± 4.2% (table 1). Ropivacaine reduced the rolling fraction by half. In the second series in the same preparations, ropivacaine significantly reduced the spontaneous leukocyte rolling flux and the rolling fraction by 57% and 47%, respectively (data not shown).

In postcapillary venules, the base-line rolling flux was 45.0 ± 11.0 cells/min, and ropivacaine reduced this rolling by 96% (table 1). The effects of ropivacaine were found to be reversible. That is, after washout of applied LTB₄ and ropi-
preparation (data not shown).

flux by 35% on repetition of the experiments in the same
by 47%. Ropivacaine significantly reduced the total leukocyte
61.8
shown in table 1. The base-line total leukocyte flux was
ing and rolling leukocytes, a value that reflects blood flow, is
6
itself inhibited the spontaneous rolling leukocyte flux (P
,
, signifi-
4.4% (P
,
, n
5
for 15 min significantly reduced arteriolar diameters by 43%

LTB4, the peak number of leakage sites was markedly re-
duced (fig. 3). The effect was fully reversible on washout after
10 µM ropivacaine, but it was only partially reversible after
100 µM.

**Leukocyte expression of L-selectin.** L-selectin is rap-
idly shed by proteolytic cleavage after leukocyte activation
(Kishimoto et al., 1989). Accordingly, the granulocyte mem-
brane expression of L-selectin was decreased by 50% (from
13 ± 1.8 to 6.5 ± 0.3) after 15 min of activation (TNF-α) as
compared with controls. Ropivacaine and lidocaine dose-de-
pendently suppressed L-selectin shedding, the lowest concen-
trations with significant effects being 100 and 300 µM, re-
spectively, (fig. 4). In addition, ropivacaine inhibited the
shedding of L-selectin on monocytes. However, this effect was
not so pronounced as that seen for granulocytes (data not
shown). Lidocaine was inactive in this regard.

**Intracellular Ca”” concentrations.** Stimulation of leu-
kocytes with LTB4 (0.01, 0.1 and 1 µM) induced a dose-
dependent rise in [Ca””]i—from a basal level of 115 nM to
290, 350 and 405 nM, respectively (n = 4). Pretreatment of
the leukocytes for 10 min with 1 mM ropivacaine did not
affect the LTB4-induced Ca”” transients significantly, i.e.,
from 175 nM to 350, 400, and 465 nM, respectively.

**Discussion**

A key event in inflammation is the recruitment of leuko-
cytes to sites of inflammation. This recruitment consists of
several sequential steps, including leukocyte rolling along
the endothelium followed by firm adhesion of the leukocytes
to the endothelial cells. In the present study, we show that
ropivacaine can reduce both the rolling and the firm adhesion
in vivo, as well as the increased vascular permeability asso-
ciated with leukocyte adhesion. Furthermore, ropivacaine
was found to inhibit the induced expression of CD11b/CD18
on leukocytes in vitro.

Ropivacaine almost completely inhibited the LTB4-induced
leukocyte adhesion in both postcapillary and larger venules.
This may have been the result of a direct effect on firm
adhesion and/or, given that venular rolling is a prerequisite
for firm adhesion (Lindbom et al., 1992), an indirect effect
mediated through inhibition of leukocyte rolling. Because
LTB4 has been shown to stimulate leukocyte adhesion
through CD11b/CD18 (Arfors et al., 1987; Tonnesen et al.,
1989), our in vitro finding that ropivacaine inhibited up-
regulation of neutrophil CD11b/CD18 indicates that the ob-
served effect of ropivacaine on adhesion was, at least in part,
mediated through reduction of CD11b/CD18 expression.
Interestingly, it has been suggested that CD11b/CD18 is in-
volved in the interactions between intestinal epithelial cells
and neutrophils (Parkos et al., 1995), which implies that
ropivacaine may also interfere with transepithelial leukocyte
migration.

The mechanism by which the local anesthetics inhibited
expression of CD11b/CD18 is unknown. However, because
local anesthetics are known to act on ion channels to decrease
membrane permeability to Na”” and K”” in nerves and may
have a similar action on other cell types, one possibility is
that local anesthetics interact with different ion channels on
the leukocytes. Leonard et al. (1992) have found that the
membrane potential of resting T cells is set by voltage-acti-
vated channels and that blockage of these channels is suffi-
cient to depolarize resting human T cells and prevent their
ability (1). Starting 10 min before the second and fourth LTB4 applications, ropivacaine (black bars) was added to the superfusate at a final concentration of $10^{-5}$ M (2) and $10^{-6}$ M (4), respectively. The ropivacaine-mediated inhibition was reversible as shown by LTB4 challenge during the washout periods (3) and (5). The results are mean values ± S.E.M. in six hamsters. *P < .05 compared with control value. **P < .01. ***P < .001 as compared with the first LTB4 application (1).

The inhibition of the rolling leukocyte fraction was related to L-selectin shedding. Values are means ± S.E.M. *P < .05, **P < .01.

However, the results so far do not support a hypothesis of an action on leukocyte calcium mechanisms. It could also be speculated that the effect on adhesion is due to inhibition of endogenous LTB4. This is unlikely, however, because 5-lipoxygenase inhibitors have been shown to be ineffective in acute (as in this study) leukocyte-dependent LTB4-induced responses in the hamster cheek pouch (Raud, 1989).

The close relationship between initial leukocyte rolling flux and subsequent adhesion (Lindbom et al., 1992; Mayrovitz, 1992) suggests another mechanism by which ropivacaine might inhibit adhesion: inhibition of leukocyte rolling. We found that ropivacaine reduced venular rolling leukocyte flux in vivo by 70%, and, in contrast to the LTB4-mediated effect, the effect of ropivacaine was not due to increased adhesion. Inhibition of the rolling flux may be the result of a reduced fraction of rolling leukocytes and/or reduced delivery of leukocytes (i.e., blood flow). Ropivacaine was found to inhibit the rolling leukocyte fraction by approximately 50% compared with the control condition. Because the magnitude of the rolling leukocyte fraction is dependent on the selectins and/or their ligands (Ley and Tedder, 1995), the latter finding indicates that ropivacaine somehow interfered with selectin expression or function. It has been demonstrated that the spontaneous leukocyte rolling observed after preparation of tissues for intravital microscopy is mediated by both L- and P-selectin (Todd et al., 1984; Doré, et al., 1993; von Andrian et al., 1991). However, it is unlikely that the inhibitory effect of ropivacaine on the leukocyte rolling was related to L-selectin expression, because treatment with 100 μM ropivacaine retained surface expression of L-selectin on the granulocytes. This leaves endothelial P-selectin as a possible target of ropivacaine. Furthermore, local anesthetics have been suggested to “stabilize” the cell membrane of leukocytes (Young and MacKenzie, 1992), and L-selectin and the P-selectin glycoprotein ligand-1 are localized on the microvilli of neutrophils to improve the presentation of these molecules to the endothelium (Patel et al., 1995). Therefore, it is possible that ropivacaine reduces rolling by changing leukocyte cell membrane morphology and adhesion molecule distribution.

The inhibition of the rolling leukocyte fraction accounted for approximately 50% of the effect of ropivacaine on the rolling leukocyte flux. The remaining effect by ropivacaine on the rolling flux appeared to be related to a reduction in blood flow, detected as a partial arteriolar constriction and as a significant reduction in the total leukocyte flux (which reflects blood flow), a value that is known to be correlated with

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**Table 1**

Effects of ropivacaine and leukotriene B4 on microvascular leukocyte kinetics in the hamster cheek pouch

<table>
<thead>
<tr>
<th>First Series</th>
<th>Leukocyte Adhesion (cells/10,000 μm²)</th>
<th>Rolling Leukocyte Flux (cells/min)</th>
<th>Total Leukocyte Flux (cells/min)</th>
<th>Leukocyte Rolling Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.2 ± 3.6</td>
<td>26.6 ± 6.9</td>
<td>61.8 ± 1.6</td>
<td>41.0 ± 4.2</td>
</tr>
<tr>
<td>LTB4</td>
<td>18.2 ± 1.5*</td>
<td>7.4 ± 1.7*</td>
<td>32.8 ± 6.5*</td>
<td>21.8 ± 3.2*</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>7.2 ± 1.7</td>
<td>2.0 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTB4 + ropivacaine</td>
<td>8.4 ± 1.5*</td>
<td>16.0 ± 2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were obtained from experiments described in figure 2. Values are expressed as means ± S.E.M. during a 2-min control period immediately before LTB4 challenge and at the last 2 min of ropivacaine and/or LTB4 application either in venules (bold) or postcapillary venules (italics). For clarity, data of no relevance to the effect of ropivacaine on LTB4-induced adhesion are replaced by dash (—).

*P < .05 compared with control value. **P < .01 compared with LTB4-induced value.

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**Fig. 3.** Number of postcapillary leakage sites per square centimeter after five repeated applications of 10 nM LTB4 for 5 min to the hamster cheek pouch. The first application of LTB4 greatly increased permeability (1). Starting 10 min before the second and fourth LTB4 applications, ropivacaine (black bars) was added to the superfusate at a final concentration of 10 μM (2) and 100 μM (4), respectively. The ropivacaine-mediated inhibition was reversible as shown by LTB4 challenge during the washout periods (3) and (5). The results are mean values ± S.E.M. in six hamsters. *P < .01, **P < .001 as compared with the first LTB4 application (1).

**Fig. 4.** Dose-response curves of L-selectin expression on human granulocytes (n = 6 for ropivacaine and n = 8 for lidocaine). Cells were incubated with TNF-α (10^{-10} g/ml) and with ropivacaine (■) or lidocaine (□), respectively, for 15 min at 37°C. Incubation with TNF-α alone was used as control. Ropivacaine and lidocaine dose-dependently inhibited L-selectin shedding. Values are means ± S.E.M. *P < .05, **P < .01.
the rolling leukocyte flux (Thorlacius et al., 1995). We thus suggest that the inhibitory effect by ropivacaine on leukocyte rolling was partly due to changes in leukocyte-endothelium adhesive interactions and partly related to alterations in blood flow.

Ropivacaine markedly inhibited the LTβ- induced plasma leakage in a dose-dependent and reversible manner. Because LTβ- induced plasma extravasation is mediated by leukocytes (Björk et al., 1982; Kurose et al., 1994), the inhibition of plasma leakage by ropivacaine may be a result of its ability to reduce leukocyte-endothelial cell interactions.

The inhibitory effect of ropivacaine on leukocyte adhesion differs from the anti-inflammatory action of glucocorticoids, which are commonly used for the local treatment of UC. In contrast to ropivacaine, glucocorticoids do not inhibit the increased endothelial adhesion induced by chemotactic factors but instead inhibit the leukocyte extravasation process (Oda and Katori, 1992). Interestingly, metronidazole, a potential antimicrobial agent that is gaining recognition as a possible mode of therapy for treatment of UC, has effects comparable to those of ropivacaine. This agent has been shown to inhibit LTβ- induced adhesion in the microcirculation of the rat mesentery (Arndt et al., 1994). With regard to lidocaine, another drug tested for treatment of UC, our study confirms previous observations that this local anesthetic can inhibit CD11b/CD18 up-regulation and L-selectin down-regulation on neutrophils (Ohsaka et al., 1994). However, we found that lidocaine was 2.5 times less potent than ropivacaine in inhibiting the CD11b/CD18 expression.

In conclusion, ropivacaine was found to inhibit inflammatory leukocyte rolling, firm adhesion and the associated increased vascular permeability in vivo. Moreover, our in vitro findings showed that ropivacaine had an inhibitory effect on induced expression of CD11b/CD18. Because leukocyte-endothelial cell interactions represent early and rate-limiting steps in intestinal inflammatory processes, these findings may help explain the beneficial effect of ropivacaine seen in the treatment of UC.

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