Beneficial Effects of Ropivacaine in Rat Experimental Colitis

TITTI MARTINSSON, TRYGGVE LJUNG, CARLOS RUBIO, and PER M. HELLSTROM

ABSTRACT

Ropivacaine, a new, long-acting local anesthetic agent, has been shown to have beneficial effects in the treatment of ulcerative colitis. Treatment with this drug results in prompt symptomatic relief. The aim of this study was to examine the effects of ropivacaine on mucosal healing and to investigate whether ropivacaine can restore the decreased colonic contractility seen in the diseased state. Colitis was induced in rats by a single intrarectal administration of trinitrobenzene sulfonic acid. Mucosal healing was assessed after 1 week of therapy. The effects on colonic contractility were examined either after 1 week of treatment or by application of the drugs to untreated, inflamed rat colon segments placed in organ baths. After the induction of colitis, daily intracolonial treatment with ropivacaine for 1 week reduced morphological damage and myeloperoxidase activity. One week of treatment also restored the contractile response to acetylcholine. By adding ropivacaine directly to untreated inflamed colonic segments in organ baths, the contractile response to acetylcholine was increased compared with controls. For comparison, the effects of budesonide and 5-aminosalicylic acid were also examined. Ropivacaine improved mucosal healing and restored colonic motor activity in experimental colitis, similar to budesonide but superior to 5-aminosalicylic acid.

Materials and Methods

Animals

Male Sprague-Dawley rats (B&K Universal, Sollentuna, Sweden) weighing 200 to 300 g were used. The animals were kept in a restricted access room with controlled temperature (23°C) and light/dark cycle (12 h/12 h). The rats were housed in individual standard wire mesh cages. Food and water were provided ad libitum. The study was approved by the local animal ethics committee.

Experimental Colitis

Experimental colitis was induced using TNBS as described by Morris et al. (1989) with slight modifications. In brief, rats fasted for 12 h were anesthetized i.m. with Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen Animal Health, Bersee, Belgium), and a baby-feeding tube was inserted rectally into the colon so that the tip was 8 cm proximal to the anus. Thereafter, 0.60 mg of TNBS in 0.2 ml of saline was administered into the rectum. The rats were randomized into six groups: control, ropivacaine (10 mg/ml), budesonide (1 mg/ml), 5-ASA (2 mg/ml), budesonide + ropivacaine, budesonide + 5-ASA, and budesonide + ropivacaine + 5-ASA. The rats were treated daily for 1 week.

ABBREVIATIONS: UC, ulcerative colitis; ACh, acetylcholine; $E_{max}$, maximal contractile response; LTB$_4$, leukotriene B$_4$; 5-ASA, 5-aminosalicylic acid; MPO, myeloperoxidase; TNBS, trinitrobenzene sulfonic acid.
ml of TNBS 5% w/v (40 mg; Sigma Chemical Co., St. Louis, MO) in 0.25 ml of 50% ethanol, resulting in a total volume of 0.85 ml, was instilled into the lumen of the colon, and the tube was flushed with 0.5 ml of air. In prestudy experiments, we found that our composition of the TNBS solution gave a prompt inflammation with uniform spread along the colon with minimal mortality rate. Because this provocation resulted in stable conditions in terms of inflamed surviving animals, we chose this slightly different TNBS solution.

**Experimental Design**

TNBS/ethanol colitis was induced in 33 rats randomized into four groups to receive no therapy (n = 8) or daily rectal administration of 0.2 ml ropivacaine (8 mg/kg, n = 10; Astra, Södertälje, Sweden), budesonide (0.1 mg/kg, n = 8; Astra Draco, Lund, Sweden; Ekström, 1998), or 5-aminosalicylic acid (5-ASA; 120 mg/kg; n = 7; Sigma Chemical Co.; Wallace et al., 1989) from day 1 (24 h after the induction of colitis) to day 7 when the animals are sacrificed. In addition, sham colitis with intrarectal saline was induced in 10 rats (control group). The concentrations of ropivacaine are within the therapeutic range obtained in the distal colon of patients treated with rectal administration of the drugs (Arlander et al., 1996).

**Tissue Preparation**

After 1 week, experimental and control rats were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Hundelein, IL), and the distal colon (10 cm) was rapidly removed and placed in Krebs’ bicarbonate solution (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 2.5 mM CaCl2, 25 mM NaHCO3, 11 mM glucose, and 0.1% bovine serum albumin). Three representative specimens (rings measuring 2–4 mm in length) were obtained from the colon from a region 2 to 4 cm proximal to the anus. One segment was taken for motility experiments. Another specimen was frozen in 0.05 M phosphate buffer, pH 6, containing 0.5% hexadecyl-trimethylammonium bromide for myeloperoxidase (MPO) and nitrite/nitrate analysis. A third sample was fixed in 4% formaldehyde for routine histological examination. The remaining portion of the colon was assessed macroscopically for mucosal damage.

**Colonic Inflammation**

**Macroscopic and Histological Evaluation.** The colonic damage was scored on a scale of 0 to 5 as described by Morris et al. (1989). In addition, the presence or absence of intra-abdominal adhesions was noted, together with the presence or absence of diarrhea, defined as loose, watery stools. Tissues taken for histological evaluation were embedded in paraffin wax. Sections (4 μm) were stained with hematoxylin-eosin and evaluated by light microscopy for ulceration and transmural inflammation in a blinded fashion.

**MPO and Nitrite/Nitrate Analysis.** Full-thickness tissue was homogenized and the MPO activity was measured as described previously (Schierwagen et al., 1990). The total nitrite/nitrate concentrations as final products of nitric oxide (NO) in the tissues were measured using a fluorometric assay (Cayman Chemical Co., Ann Arbor, MI).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Changes in body weight, colonic wet weight, diarrhea, and adhesions to other organs 1 week after rectal trinitrobenzene sulfonic acid administration: Effects of ropivacaine, 5-aminosalicylic acid, and budesonide</th>
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<tbody>
<tr>
<td>Increase in Body Weight</td>
<td>Colonic Wet Weight</td>
</tr>
<tr>
<td><strong>g</strong></td>
<td>mg/cm</td>
</tr>
<tr>
<td>TNBS</td>
<td>1.1 ± 2.2*</td>
</tr>
<tr>
<td>TNBS + ropivacaine</td>
<td>13.2 ± 13.2</td>
</tr>
<tr>
<td>TNBS + budesonide</td>
<td>22.2 ± 16.6*</td>
</tr>
<tr>
<td>TNBS + 5-ASA</td>
<td>5.7 ± 11.6</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. (body weight and colonic wet weight), number of rats with diarrhea, or adhesions/total number of rats in each group. The drugs were administered daily for 1 week starting 24 h after the induction of colitis with TNBS.

* p < .001 compared with the normal group. † p < .05 and ‡ p < .01 compared with the inflamed untreated group. The increase in body weight for normal rats was 62.9 ± 18.3 g, the colonic wet weight was 190 ± 10 mg/cm, and these rats showed no signs of diarrhea or bowel adhesions (n = 10).

**Colonic Contractility.** Longitudinal colonic segments were mounted in 5-ml chambers containing Krebs’ bicarbonate solution kept at 37°C and gassed with 5% CO2 in O2. The segments were equilibrated for 30 min and then maintained at a tension of 9.81 mN for 1 h with repeated washings. Isometric contraction was measured with a Grass FT03C force displacement transducer (Grass Instrument Co., Quincy, MA) with a Grass Polygraph 7B.

Functional recovery of inflamed colon after 1 week of treatment was examined as contractile response to ACh in inflamed versus treated tissue and compared with the control group consisting of rats with sham colitis. The segments were washed after each exposure to ACh and left for 5 min to recover baseline tension before the next application. ACh was added in a volume of less than 1% of the bath volume.

To investigate the direct effects of ropivacaine, budesonide, and 5-ASA on contractile responses of inflamed colon, the response to ACh was measured before and after (10 min) drug application (in vitro) to inflamed colonic segments. TNBS/ethanol colitis was induced in 30 rats, and sham colitis was induced in 5 rats. These rats received no therapy. The drugs were tested at concentrations of 1 mM (ropivacaine), 0.8 mM (budesonide), and 3 mM (5-ASA). The contractile responses were expressed as a percentage of the maximal response to ACh of normal colons. The maximal contractile response, Emax, was calculated for each treatment. Concentration-response curves were fitted to a nonlinear regression model.

**Statistical Analysis**

All values are expressed as the mean ± S.E. The Kruskal-Wallis test followed by the Mann-Whitney U test or the Wilcoxon signed rank test (for contractility data) was used for statistical evaluation. A value of p < .05 was chosen as the level of significance.

**Results**

Intracolonic administration of TNBS/ethanol resulted in an inflammatory response characterized by extensive mucosal disruption, linear and deep ulcers, hemorrhage, and submucosal edema. Intra-abdominal pathological adhesions between colon and small bowel and other organs were seen in 50% of the rats. Diarrhea and lack of weight gain were evident in all rats without treatment (Table 1).

**Effects of Drug Treatment on Colonic Inflammation**

**Macroscopic Damage.** Daily intracolonic treatment with ropivacaine, budesonide, or 5-ASA for 7 days attenuated the macroscopic damage (Fig. 1A) and reduced MPO activity (Fig. 1B). The beneficial effects of ropivacaine were also evident because colonic wet weight was reduced by almost 50% in ropivacaine-treated compared with untreated rats. By way of comparison, 5-ASA reduced colonic wet weight, but budesonide did not. The prevalence of diarrhea was reduced by more than 50% in the ropivacaine and budesonide groups,
whereas the 5-ASA treatment had no effect. The number of adhesions between the colon and other organs, as well as the body weight, were not significantly affected by the drug treatments (Table 1). In sham-treated rats, no macroscopic damage was observed.

**Histological Score**

Histological assessment showed transmural inflammation and ulceration in inflamed untreated rats. In addition, extensive polymorphonuclear granulocyte infiltration was apparent. The drug-treated groups showed little histological improvement (Fig. 2). Mucosal integrity was not restored in any of the inflamed animals. However, ulceration and transmural inflammation were reduced in three of nine ropivacaine-treated rats and in two of six and one of seven rats treated with budesonide and 5-ASA, respectively.

**MPO Activity and Nitrite/Nitrate Concentrations**

In normal rats, basal MPO activity was $6 \pm 1 \text{U/g}$. This value increased 15-fold in the TNBS-treated rats ($p < .001$). All drug treatments significantly reduced MPO activity (Fig. 1B). Inflamed tissue contained increased amounts of nitrite/nitrate compared with noninflamed tissue (34 ± 8 versus 24 ± 7 nmol/g). Tissue treated with ropivacaine, budesonide, and 5-ASA contained 35 ± 13, 42 ± 10, and 36 ± 15 nmol/g, respectively, which was not different from inflamed controls. However, after budesonide treatment, the nitrite/nitrate lev-
els were significantly increased compared with normal non-inflamed tissue ($p < .05$).

**Effects of Drug Treatment on Colonic Contractility**

**Functional Recovery.** ACh caused concentration-dependent contractions of the noninflamed colonic segments ($E_{\text{max}} = 64 \pm 25 \text{ mN}$). The contractile response of the inflamed segments was decreased ($E_{\text{max}} = 16 \pm 8 \text{ mN}; 25 \pm 13\%$). Ropivacaine and budesonide treatment during 1 week increased the response to ACh in inflamed segments to a level similar to normal colon, whereas 5-ASA only slightly increased contractions in response to ACh (Fig. 3).

**Direct Effects.** ACh-induced contractions in inflamed colon were markedly decreased ($E_{\text{max}} = 18 \pm 20\%$) compared with normal colon. The addition of ropivacaine in vitro 10 min before ACh increased the contractile response compared with inflamed controls (Fig. 4), whereas the normal colonic motility response was unaffected by the drug (data not shown). Preincubation with budesonide or 5-ASA did not affect the reduced contractile response to ACh in inflamed tissue (data not shown).

**Discussion**

In the present study, treatment with ropivacaine or budesonide markedly reduced the inflammation in a rat model of colitis, as verified by effects on tissue macroscopic damage and MPO activity. The contractile response of inflamed colon was attenuated compared with noninflamed colon. The treatment with ropivacaine or budesonide potently enhanced colonic contractions in response to ACh. This effect was already apparent after a 10-min incubation period with ropivacaine in vitro, whereas budesonide and 5-ASA had no effects under these circumstances. This indicates that the ropivacaine-mediated stimulation of contractility is not merely a result of tissue healing but also a direct effect of ropivacaine on the tissue.

Our results show a beneficial effect of ropivacaine on the inflammatory response in an animal model of colitis. One week of treatment with ropivacaine reduced the colonic mucosal inflammation as demonstrated by macroscopic examination, colonic wet weight, prevalence of diarrhea, and MPO activity. This is in agreement with findings of McCafferty et al. (1994, 1997), who showed similar anti-inflammatory effects of lidocaine, suggesting that the drug attenuates colitis by acting on enteric nerves. However, the mechanisms by which ropivacaine exerts its anti-inflammatory action appear to be more complex. In addition to possible effects on the enteric nerves, ropivacaine may have direct anti-inflammatory actions, as indicated by effects on leukocyte rolling and adhesion in vivo (Martinsson et al., 1997b). Neutrophil recruitment contributes to the pathology of different inflammatory diseases such as UC (Babbs, 1992), where suppression of neutrophil function has also been shown to reduce tissue damage (Palmen et al., 1995). The neutrophils release large amounts of inflammatory mediators, including LTB$_4$. Specific 5-lipoxygenase inhibitors and LTB$_4$ receptor antagonists have been shown to reduce intestinal inflammation (Fretland et al., 1989; Bertrán et al., 1996) and colonic myoelectrical disturbances induced by TNBS (Morteau et al., 1993). We have previously shown that ropivacaine inhibits LTB$_4$ release from granulocytes (Martinsson et al., 1997a), which may contribute to the anti-inflammatory action of ropivacaine seen in our present report.

Along with free oxygen radicals, NO-derived metabolites may contribute to tissue dysfunction in inflammation (Alican and Kubes, 1996), and luminal concentrations of NO are greatly increased in UC (Lundberg et al., 1994). NO induces smooth muscle relaxation and colonic dilatation (Moureille et al., 1995). Rectal dialysates from patients with UC contain high levels of nitrate (Roediger et al., 1986). We found increased levels of nitrite/nitrate in inflamed rat colonic tissue; however, the treatment groups showed similar levels despite improved colonic motility. Thus, the results are inconclusive and do not indicate whether the treatments in this study mediate the effects on motility by affecting NO. The finding that budesonide treatment increased nitrite/nitrate levels in the tissue compared with the levels in the normal tissue has yet to be explained. However, similar effects have previously been observed with $\text{N}^\bullet$-nitro-L-arginine methyl ester and $\text{N}$-monomethyl-L-arginine producing a dual response with an immediate enhancement of NO levels shifting to an inhibition over time (Laszlo et al., 1994).

The potentiating effect of ropivacaine on colonic contraction in response to ACh may result from a direct effect on smooth muscle cells or an indirect effect through enteric neurons. Likewise, the colonic dilatation in patients with UC...
may be due to the inflammatory reaction impeding myogenic function or neural control (Snape et al., 1980). In vitro studies of circular colonic muscle from patients with UC suggest a muscle defect at a biochemical level, resulting in reduced force development (Snape et al., 1987). It has previously been suggested that local anesthetic agents have a direct excitatory effect on smooth muscle cells (longitudinal and circular preparations; Bortoff and Muller, 1975) because these agents can stimulate responses of the intact intestinal wall, as well as ganglion-free muscle preparations. K+-induced membrane depolarization has been shown to induce contraction of smooth muscle (Nielsen-Kudsk, 1996). This effect is due to transmembrane Ca2+ influx, which initiates the contraction process (Snape and Tan, 1985). We have seen that ropivacaine can directly depolarize human colon adenocarcinoma cells (Martinsson, 1999). A direct excitatory effect may therefore be explained in these terms.

An indirect stimulatory effect of ropivacaine due to actions on enteric neurons could be mediated through the blockade of a toxic inhibitory neuronal input, leading to a greater responsiveness to contractile stimulation. In support of this, Wood (1972) showed that lidocaine produces action potentials and increases the amplitude of contractions in intestinal muscle. In further support of this hypothesis, intra-arterial injections of lidocaine and procaine in an extrinsically denervated intestinal vascular bed have been shown to increase intestinal motility (Biber and Fara, 1973), most likely as a result of suppression of intrinsic nervous inhibition.

Second, ropivacaine may influence the production of mediators that decrease colonic contraction. It is well established that the mucosal levels of several prostaglandins and leukotrienes are elevated in UC (Sharon and Stenson, 1984), and members of the arachidonic acid pathway have long been known to modulate intestinal motility in humans and various animal species (Bennett et al., 1981). One example is LTB4, which has a relaxatory effect on intestinal contractility in vitro (P. Hellström, unpublished observation). Because ropivacaine can inhibit LTB4 from human granulocytes (Martinsson et al., 1997a), it is conceivable that ropivacaine may interfere with such mechanisms to increase contractile responses in the gut.

In conclusion, our results show that ropivacaine therapy in experimental colitis improved mucosal healing and restored intestinal motor activity. It is possible that the marked reduction in the contractility of inflamed colonic muscle contributes to the overall reduction in motor activity observed in acute colitis. Thus, the improvement of colonic motility mediated by ropivacaine may contribute to the prompt symptomatic relief seen in the patients with UC.

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References


Fig. 4. Isometric tension development in colonic longitudinal muscle 1 week after induction of colitis, before (●) and 10 min after (○) the addition of ropivacaine (1 mM). The curves were fitted according to a nonlinear regression model using the least-squares method. *p < 0.05, significant difference from the inflamed untreated group. Values are mean ± S.E. (n = 8).


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