Beneficial Effects of Ropivacaine in Rat Experimental Colitis

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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 intracolon 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/ml 2,4,6-trinitrobenzene sulfonic acid was administered for 2 weeks.

Materials and Methods

Ropivacaine, a new local anesthetic agent currently under investigation for the treatment of ulcerative colitis (UC), improves inflammatory endoscopic scores after only 2 weeks of treatment (Arlander et al., 1996). In experimental studies, ropivacaine has been found to inhibit leukocyte adhesion and expression of adhesion molecules (Martinsson et al., 1997b) and the formation of leukotriene B4 (LTB4) and reactive oxygen metabolites (Cederholm et al., 1994; Martinsson et al., 1997a) from human neutrophils. These observations are in line with several previous studies indicating anti-inflammatory effects of local anesthetic agents other than ropivacaine (Cullen and Haschke, 1974; MacGregor et al., 1980; Sinclair et al., 1993).

Inflammation of the gastrointestinal tract results in the disturbance of motility in humans and in animal models. The disruption of normal motor patterns in intestinal inflammation, including colonic hypomotility and unresponsiveness to contractile stimuli, contributes to commonly observed clinical features, such as diarrhea (Spriggs et al., 1951; Snape et al., 1980; Wallace et al., 1991). The changes in colonic motility and secretion may reflect alterations in the function of smooth muscle or the enteric nervous system, or both. Restoration of colonic motility may perhaps explain the observation that daily intrarectal administration of local anesthetic agents commonly results in symptomatic relief (within a few days) in patients with UC (Björck et al., 1993).

The aim of this study was to investigate the contractile responses in vitro of normal and inflamed rat colon and to investigate the effects of ropivacaine on contractions of colonic smooth muscle induced by acetylcholine (ACh). Furthermore, we examined the ability of ropivacaine to modify the course of mucosal pathology in experimental colitis induced in the rat with trinitrobenzene sulfonic acid (TNBS).

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ABBREVIATIONS: UC, ulcerative colitis; ACh, acetylcholine; \( E_{\text{max}} \), maximal contractile response; LTB4, leukotriene B4; 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 addition, sham colitis with intrarectal saline was induced in 10 rats (control group). The concentrations of ropivacaine are within the

**Tissue Preparation**

After 1 week, experimental and control rats were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Huddlein, 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.

**Colon 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 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Increase in Body Weight (g)</th>
<th>Colonic Wet Weight (mg/cm)</th>
<th>Diarrhea</th>
<th>Adhesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBS</td>
<td>1.1 ± 2.2*</td>
<td>480 ± 200*</td>
<td>2/10</td>
<td>4/10</td>
</tr>
<tr>
<td>TNBS + ropivacaine</td>
<td>12.2 ± 12.0</td>
<td>270 ± 55*</td>
<td>3/8</td>
<td>4/10</td>
</tr>
<tr>
<td>TNBS + budesonide</td>
<td>22.2 ± 16.6*</td>
<td>320 ± 100</td>
<td>2/8</td>
<td>6/7</td>
</tr>
<tr>
<td>TNBS + 5-ASA</td>
<td>5.7 ± 11.6</td>
<td>280 ± 95*</td>
<td>6/7</td>
<td>6/7</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.

**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 ± 1 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 LTB4. Specific 5-lipoxygenase inhibitors and LTB4 receptor antagonists have been shown to reduce intestinal inflammation (Fretland et al., 1989; Bertran et al., 1996) and colonic myoelectrical disturbances induced by TNBS (Morteau et al., 1993). We have previously shown that ropivacaine inhibits LTB4 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 (Mourelle et al., 1995). Rectal dialysates from patients with UC contain high levels of nitrite (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 N6-nitro-L-arginine methyl ester and 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

**Fig. 3.** A, isometric tension development in colonic longitudinal muscle from normal rats (■) or 1 week after the induction of colitis (○). Tension was stimulated by ACh, and the results are expressed as a percentage of the maximal ACh response of normal colon. B, effects of ropivacaine (●), budesonide (▲), and 5-ASA (★) after 1 week of treatment compared with untreated colon (□). The curves were fitted according to a nonlinear regression model using the least-squares method. *$p < .05$, **$p < .01$, significant difference from the inflamed untreated group. Values are mean ± S.E. ($n = 7$ rats/group).
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 (Nielson-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 tonic 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 < .05, significant difference from the inflamed untreated group. Values are mean ± S.E. (n = 8).


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