Anti-inflammatory efficiency of levobupivacaine in an experimental colitis model

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Abstract

AIM: To investigate the efficiency of levobupivacaine in treating experimentally induced colitis in rats.

METHODS: Colitis was induced by trinitrobenzene sulfonic acid and ethanol in 30 rats under general anesthesia, and 10 rats were used as a sham group. Subsequent to induction of colitis, rats were divided into three groups; budesonide group received 0.1 mg/kg budesonide, levobupivacaine group received 10 mg/kg levobupivacaine and saline group received 1 mL saline solution via rectal route for 7 d. In the sham group, only routine rectal catheterization was performed without use of any material. At the end of 7 d, laparotomy and total colectomy were performed for histopathological examination in all rats and blood samples were drawn for measurement of tumor necrosis factor (TNF)-α and interleukin (IL)-6 following cardiac puncture. Macroscopic and microscopic evaluations of the specimens were performed by a pathologist blinded to group assignment of the rats.

RESULTS: Weight loss ($P = 0.016$) and macroscopic examination scores ($P = 0.001$) were significantly higher in saline group than others. Histopathological scoring was comparable between all colitis groups ($P = 0.350$). There was no significant difference in TNF-α levels and IL-6 levels ($P = 0.150$).

CONCLUSION: The significant improvement in macroscopic scores suggests that levobupivacaine may have topical anti-inflammatory effects in an experimental colitis model; however, this finding was not supported by microscopic findings.

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Key words: Trinitrobenzene sulfonic acid; Colitis; Levobupivacaine; Budesonide

INTRODUCTION

Inflammatory bowel disease (IBD), usually referring to
ulcerative colitis or Crohn’s disease, has an unknown etiology[1]; however, some theories have been proposed. One of the key factors is the excessive immune response of the host[2], which has led to broad use of corticosteroids[3]. Potentially serious side effects of corticosteroids are the most important limiting factor[4]. The autonomic imbalance between sympathetic and parasympathetic nerves is anticipated as another etiological factor[5]. Adrenergic preponderance had been found to be associated with increased epithelial turnover and vasoconstriction resulting in mucosal injury[6]. The beneficial effects of nicotine on IBD support this theory[7].

Local anesthetics, particularly lidocaine, have inhibitory effects on hyperactive autonomic nerves and on host immune response[8,9]. When all these effects are put together, it is not surprising that local lidocaine was effective in the treatment of experimentally produced IBD[10]. Ropivacaine, another local anesthetic with longer acting time than lidocaine had a more pronounced protective effect on mucosal damage when it was topically applied to colonic mucosa in comparison to 5-amino salicylic acid (5-ASA)[10-13]. In this study, we investigated the potential of levobupivacaine, which is a novel, long lasting local anesthetic with less systemic side effects[14], in the treatment of experimentally induced IBD.

**MATERIALS AND METHODS**

All procedures in this study were approved by local animal ethics committee. The histopathological examinations and biochemical studies were performed, respectively in research laboratories of pathology and biochemistry at Uludag University School of Medicine.

Forty male Sprague-Dawley rats weighing 270 to 390 g were used in this study. The animals were kept in a restricted access room with controlled temperature and light cycle. All rats were housed in individual standard cages. *Ad libitum* standard pellet food and water were maintained for all animals to provide minimum stress.

**Induction of colitis**

At the day of induction all rats were anesthetized with intramuscular ketamine (8 mg/kg, Ketalar®, Pfizer Inc.) and xylazine (90 mg/kg, Ronpum®, Bayer AG). A 6F feeding tube was inserted rectally until the tip was 8 cm proximal to the anus. The mixture of 0.6 mL of trinitro-benzene sulfonic acid (TNBS) (5% w/v; 40 mg, Sigma Chemical Co., St. Louis, USA) and 0.25 mL of 50% ethanol was instilled into the lumen via a feeding tube. Finally, 0.5 mL of air was given to ensure that the whole mixture was instilled into the lumen of the colon.

There were four groups consisting of 10 rats for each: (1) Sham group: no colitis was induced; only rectal insertion of feeding tube was performed once a day from day 1 to day 7; (2) Budesonide group: rats were treated with daily rectal single dose of budesonide (0.1 mg/kg, Entocort® enema, AstraZeneca) via feeding tube for 7 d following the induction of colitis; (3) Levobupivacaine group: rats were treated with daily rectal single dose of levobupivacaine (10 mg/kg, Chirocaine®, Abbott) via feeding tube for 7 d following the induction of colitis; and (4) Saline group: rats were treated with daily rectal single dose of saline (1 mL, 0.9% NaCl) via feeding tube for 7 d following the induction of colitis.

All rectal tube applications were performed under anesthesia.

All rats were weighed daily during the study period. At the end of 7 d, laparotomy and total colectomy after inspection for adhesions were performed for all rats under general anesthesia. Finally, all rats were sacrificed by intra-cardiac puncture to get blood samples for interleukin (IL)-6 and tumor necrosis factor (TNF)-α analysis.

When tissue samples were obtained, macroscopic

### Table 1 Macroscopic scoring of mucosal damage in colitis[15]

| Macroscopic damage                                      | Score |
|---------------------------------------------------------|-------|
| Ulceration and inflammation                             | 0     |
| None                                                    | 0     |
| Local hyperemia, no ulcer                               | 1     |
| One site of ulcer not accompanied by congestion or thickening of the intestinal wall | 2     |
| One site of ulcer accompanied by inflammation           | 3     |
| Ulcer < 3 mm                                            | 4     |
| Ulcer > 3 mm                                            | 5     |
| Inflammation                                           | 6     |
| None                                                    | 0     |
| Mild (easy to separate colon from other tissues)        | 1     |
| Severe                                                  | 2     |
| Diarrhea                                                | 1     |
| Present                                                 | 1     |

### Table 2 Microscopic scoring of colitis[16]

| Histological lesion          | Score |
|------------------------------|-------|
| Ulcer                        | 0     |
| None                         | 0     |
| Ulcer < 3 mm                 | 1     |
| Ulcer > 3 mm                 | 2     |
| Inflammation                 | 3     |
| None                         | 0     |
| Mild                         | 1     |
| Severe                       | 2     |
| Granuloma                    | 1     |
| None                         | 0     |
| Present                      | 1     |
| Depth of the disease         | 5     |
| None                         | 0     |
| Submucosal layer             | 1     |
| Muscular layer               | 2     |
| Serosal layer                | 3     |
| Fibrosis                     | 5     |
| None                         | 0     |
| Mild                         | 1     |
| Severe                       | 2     |
Table 3  Comparison of study groups

|                      | Sham   | Budesonide | Levobupivacaine | Saline | P     |
|----------------------|--------|------------|-----------------|--------|-------|
| Weight loss          | 10 (0-20) | 10 (0-25) | 10 (0-20)       | 0 (0-10) | 0.016 |
| Macroscopic damage score | 0 (0-1) | 10 (0-4)   | 2 (0-5)         | 4.5 (2-9) | 0.001 |
| Microscopic damage score | 0 (0.066) | 1.33 (0-3.33) | 0 (0-5.66) | 1.66 (0-4.33) | 0.014 |
| IL-6                 | 0 (0.38-59) | 37.6 (20.3-85.4) | 32.3 (0-43.8) | 31.9 (7.7-144.8) | 0.004 |
| TNF-α                | 0.1 (0-3.7) | 0.5 (0.2-45.7) | 0.2 (0.1-312) | 0.5 (0-255.9) | 0.152 |

TNF: Tumor necrosis factor; IL: Interleukin.

DISCUSSION

In this study, we investigated whether topical administration of levobupivacaine has an anti-inflammatory efficiency in an experimentally induced colitis model. While weight alterations and macroscopic examination scores suggested that levobupivacaine might have had anti-inflammatory effects on colitis, this suggestion was not supported by histopathological findings. Although the IL-6 levels increased secondary to induction of colitis as a proof of systemic inflammatory response, diminution of IL-6 levels after levobupivacaine administration was not significant.

Weight loss in the saline group was significantly higher when compared to levobupivacaine and budesonide or sham groups (P = 0.016). This might be as a result of “no treatment” as Martinsson et al. suggested. The significant weight loss in the saline group was probably due to a more serious inflammatory process than in levobupivacaine and budesonide groups. Macroscopic damage scores also supported this finding. Levobupivacaine and budesonide groups had significantly lower macroscopic damage scores than the saline group (P = 0.001). On the other hand, macroscopic damage scores of levobupivacaine and budesonide groups were significantly higher than the sham group (P = 0.001). Treatment with levobupivacaine or budesonide had equivalently improved the mucosal damage caused by colitis. Martinsson et al. suggested that ropivacaine had more protective effects than budesonide on the mucosa in a similar study. According to our findings, levobupivacaine had similar efficiency.
with budesonide, which has been used in the treatment of clinical IBD[10]. Administration of levobupivacaine and budesonide diminished the inflammatory changes in our study, but this was not sufficient enough to draw back all the inflammatory process.

Microscopic damage scores did not show significant decreases in levobupivacaine and budesonide groups when compared to the saline group which suggests insufficient anti-inflammatory efficiency. However, we are not the only researchers who reported discordance between macroscopic and microscopic examination scores. Neither ropivacaine nor lidocaine administration decreased the microscopy scores even though they caused a significant decrease in macroscopic scores[11,17,18]. Maybe longer study periods are needed to examine the effects of topical local anesthetic treatment on IBD or maybe the microscopic scoring should be revisited.

TNF-α and IL-6 serum levels were measured to determine the systemic proofs of inflammatory response in our study. These cytokines act as proinflammatory factors in IBD and are usually related to severity of the disease[19]. In our study, IL-6 levels were significantly higher in levobupivacaine, budesonide and saline groups than in the sham group (P = 0.040). However, changes in TNF-α level did not show any significance. But it is known that TNF-α has a wide range in IBD and may sometimes not be detectable[19]. The IL-6 level in the levobupivacaine group was lower than budesonide and saline groups without statistical significance. It was reported that some local anesthetics such as lidocaine might affect the release of proinflammatory cytokines by membrane depolarization in epithelial cells[20]. The decrease of IL-6 levels in the levobupivacaine group made us think that levobupivacaine might have similar effects on cytokine levels as lidocaine. Further research might be focused on that finding.

In conclusion, despite the decrease in microscopic damage scores and IL-6 levels suggesting that topical administration of levobupivacaine might have some level of anti-inflammatory efficiency in an experimental colitis model, microscopic examination failed to support this finding. However, there may be individual differences in inflammatory response in IBD[11]. Further studies with larger groups and longer treatment periods might be beneficial in examining the potential therapeutic capacity of local anesthetics in the treatment of IBD.

REFERENCES

1. Guidi M, Riddell RH. Indeterminate colitis. J Clin Pathol 2004; 57: 1233-1244
2. Mishina D, Katsel P, Brown ST, Gilberts EC, Greenstein RJ, On the etiology of Crohn disease. Proc Natl Acad Sci USA 1996; 93: 9816-9820
3. Sands BE. Inflammatory bowel disease: past, present, and future. J Gastroenterol 2007; 42: 16-25
4. Diethelm AG. Surgical management of complications of steroid therapy. Ann Surg 1977; 185: 251-263
5. Björck S, Dahlström A, Ahlman H. Topical treatment of ulcerative proctitis with lidocaine. Scand J Gastroenterol 1989; 24: 1061-1072
6. Björck S, Dahlström A, Ahlman H. Treatment of distal colitis with local anaesthetic agents. Pharmacol Toxicol 2002; 90: 173-180
7. Calkins BM. A meta-analysis of the role of smoking in inflammatory bowel disease. Dig Dis Sci 1989; 34: 1841-1854
8. Azuma Y, Shinozawa M, Wang PL, Suese Y, Yasuda H, Ohura K. Comparison of inhibitory effects of local anesthetics on immune functions of neutrophils. Int J Immunopharmacol 2000; 22: 789-796
9. Dickstein R, Kiremidjian-Schumacher L, Stotzky G. Effect of lidocaine on the function of immunocompetent cells. IL Chronic in vivo exposure and its effects on mouse lymphocyte activation and expression of immunity. Immunopharmacology 1985; 9: 127-139
10. Lahat A, Ben-Horin S, Lang A, Fudim E, Picard O, Chowers Y. Lidocaine down-regulates nuclear factor-kappaB signaling and inhibits cytokine production and T cell proliferation. Clin Exp Immunol 2008; 152: 320-327
11. Björck S, Dahlström A, Johansson L, Ahlman H. Treatment of the mucosa with local anaesthetics in ulcerative colitis. Agents Actions 1992; Spec No: C60-C72
12. Arlander E, Ost A, Stahlberg D, Lofberg R. Ropivacaine gel in active distal ulcerative colitis and proctitis – a pharmacokinetic and exploratory clinical study. Aliment Pharmacol Ther 1996; 10: 73-81
13. Martinsson T, Ljung T, Rubio C, Hellström PM. Beneficial effects of ropivacaine in rat experimental colitis. J Pharmacol Exp Ther 1999; 291: 642-647
14. Casati A, Putzu M. Bupivacaine, levobupivacaine and ropivacaine: are they clinically different? Best Pract Res Clin Anesthesiol 2005; 19: 247-268
Duman U et al. Anti-adhesive effects of levobupivacaine

15 Wang H, Ouyang Q, Hu RW. Establishment of a trinitrobenzene sulfonic acid-induced rat colitis model. Chin J Gastroenterol 2001; 6: 7-10
16 Chan EP, Lichtenstein GR. Chemoprevention: risk reduction with medical therapy of inflammatory bowel disease. Gastroenterol Clin North Am 2006; 35: 675-712
17 Wallace JL, McCafferty DM, Sharkey KA. Lack of beneficial effect of a tachykinin receptor antagonist in experimental colitis. Regul Pept 1998; 73: 95-101
18 Chevalier E, Pétoux F, Chovet M, Langlois A. Beneficial effect of trimebutine and N-monodesmethyl trimebutine on trinitrobenzene sulfonic acid-induced colitis in rats. Life Sci 2004; 76: 319-329
19 Rogler G, Andus T. Cytokines in inflammatory bowel disease. World J Surg 1998; 22: 382-389
20 Barshack I, Levite M, Lang A, Fudim E, Picard O, Ben Horin S, Chowers Y. Functional voltage-gated sodium channels are expressed in human intestinal epithelial cells. Digestion 2008; 77: 108-117
21 MacDonald TT, Monteleone G, Pender SL. Recent developments in the immunology of inflammatory bowel disease. Scand J Immunol 2000; 51: 2-9

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