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BRIEF ARTICLE

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.

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

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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[8-10]. When all these effects are put together, it is not surprising that local lidocaine was effective in the treatment of experimentally produced IBD[11]. 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)[12,13]. 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 trinitrobenzene 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	
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
⩾ 2 sites of ulcer accompanied by inflammation	4
1 cm > inflammed segment + ulcer site ⩾ 2	5
1 cm < inflammed segment + ulcer site ⩾ 2	6
2 cm ⩽ inflammed segment (the score increases 1 with each 1 cm enlargement of the inflammed segment)	7-10
Adhesions	
None	0
Mild (easy to seperate colon from other tissues)	1
Severe	2
Diarrhea	
None	0
Present	1

Table 2 Microscopic scoring of colitis[15]

Histological lesion	Score
Ulcer	
None	0
Ulcer < 3 mm	1
Ulcer > 3 mm	2
Inflammation	
None	0
Mild	1
Severe	2
Granuloma	
None	0
Present	1
Depth of the disease	
None	0
Submucosal layer	1
Muscular layer	2
Serosal layer	3
Fibrosis	
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)	1(0-4)	2 (0-5)	4.5 (2-9)	0.001
Microscopic damage score	0 (0-0.66)	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 (0.2-255.9)	0.152

TNF: Tumor necrosis factor; IL: Interleukin.

damage was scored on a scale of 0 to 13 modified from a description by Wang *et al*[15] (Table 1) by the same pathologist who was blinded to the group assignment of the rats. Later, the tissue samples were fixed with 10% formaldehyde solution. Tissue samples of 5 mm in length were taken from proximal, middle and distal segments of total colectomy specimens. All samples were embedded in paraffin wax and sections were taken and stained with hematoxylin-eosin (HE). All sections were evaluated by light microscopy and scored on a scale of 0 to 10 as described by Wang *et al*[15] (Table 2) in a blinded fashion by the same pathologist.

Serum IL-6 and TNF-α levels were analyzed with ELISA by using rat IL-6 and TNF-α kits (Invitrogen™ Rat Immunoassay Kit; Invitrogen Corporation, Carlsbad, California, USA).

Statistical analysis

The Statistical Package for Social Sciences version 11.5 (SPSS, Chicago, IL, USA) program was used for statistical analysis of data. All values were expressed as median (minimum-maximum). The Kruskal-Wallis test followed by the Mann-Whitney *U* test was used for statistical evaluation and $P < 0.05$ was accepted as the level of significance.

RESULTS

There was no significant weight difference between budesonide, levobupivacaine and sham groups; however the weight loss in the saline group was significantly higher than other groups ($P = 0.016$).

Macroscopic damage score was significantly higher in budesonide, levobupivacaine and saline groups when compared to sham group ($P = 0.001$). No significant difference was found between budesonide and levobupivacaine groups but macroscopic damage scores of these two groups were significantly lower than saline group (Budesonide *vs* Saline: $P = 0.001$; Levobupivacaine *vs* Saline: $P = 0.002$).

Various inflammatory cell accumulations and ulcerations were the common alterations observed by light microscopy in budesonide, levobupivacaine and saline groups as there was no granuloma formation or fibrosis.

Microscopic damage scores were significantly higher in budesonide, levobupivacaine and saline groups than sham group ($P = 0.014$). Although the levobupivacaine group had lower scores than the budesonide and saline groups, there was no statistical significance (Budesonide *vs* Levobupivacaine: $P = 0.436$; Saline *vs* Levobupivacaine: $P = 0.105$).

TNF-α levels did not show any significant difference among groups ($P = 0.152$). IL-6 levels in budesonide, levobupivacaine and saline groups were significantly increased when compared to sham group ($P = 0.040$). Levobupivacaine group had slightly lower IL-6 levels than budesonide and saline group, however, there was no significant difference (Levobupivacaine *vs* Saline: $P = 0.853$; Levobupivacaine *vs* Budesonide: $P = 0.247$).

These results are summarized in Table 3.

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*[13] 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*[13] 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[16]. 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[13,17,18]. 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[21]. 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.

COMMENTS

Background

Inflammatory bowel disease (IBD) affects many people all around the world. There are several drugs that are useful in reducing the symptoms but no definitive therapeutic agents have been defined. Local anesthetics might be useful in the treatment of IBD based on their actions that inhibit hyperactive autonomic nerves and host immune response which are key factors in the pathogenesis of IBD.

Research frontiers

Previous studies reported successful results with the local application of various local anesthetics on colonic mucosa even in comparison to conventional drugs that have been officially used in the treatment of IBD for decades.

Innovations and breakthroughs

There are various types of local anesthetics. Their action period and side effects that may be seen due to the possibility of getting into the systemic blood circulation from the areas they are applied may vary. In this study, different from the previous similar studies, the authors used levobupivacaine, which is a novel, long lasting local anesthetic with less systemic side effects.

Applications

Even though there may be some side effects if they are used in high doses or applied on very wide colonic mucosa, local anesthetics are much safer than the drugs that have been conventionally used in the treatment of IBD. Therefore, if successful results are reported with the use of local anesthetics in the treatment of IBD, a safe and cheap alternative might be defined.

Peer review

The manuscript is very interesting for the readers. In their study they explored the effect of levobupivacaine and budenosine in induced colitis. The authors analyzed macro and microscopically the resected specimen and measured the serum levels of tumor necrosis factor (TNF) and interleukin (IL)-6. It could be more interesting and support more of their results if the authors could perform immunohistochemistry detection for TNF and IL-6 in the samples.

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