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ORIGINAL ARTICLE

Efficacy of Pentoxifylline and Tadalafil in Rat Model of Ischemic ColITIS

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ABSTRACT

Objective: The aim of this study is to investigate the efficacy of tadalafil against pentoxifylline in rat model of ischemic colitis (IC). *Material-Methods*: Thirty-two Wistar albino rats were subjected to laparotomy and left colon devascularization to create an IC model and then randomly placed into four groups. Group-1 (sham group) was administered 0.9% NaCl following laparotomy, group 2 (control group) was administered 0.9% NaCl following induced IC, group 3 was given pentoxifylline ($n = 8$), and group 4 was given tadalafil. On the third day; macroscopic findings, Gomella's ischemic area and Wallace scoring, histopathological analysis, and Chiu scoring were performed, and malondialdehyde (MDA) measurement in ischemic colon tissue was carried out through chemical analysis. *Results*: Significant differences were observed in acidic fluid, bowel dilatation, and serosal change ($p < .05$). The ischemic area measured 63.3 mm^2 in the control group, 2.8 mm^2 in the pentoxifylline group ($p = .0001$), and 2.4 mm^2 ($p = .0001$) in the tadalafil group. A significant difference was seen between the sham group and the control and pentoxifylline groups ($p < .01$), in terms of Wallace score and Chiu classification. Similarly, a significant difference was determined between the control group and pentoxifylline and tadalafil groups ($p < .01$), but no significant difference was established between the pentoxifylline group and tadalafil group ($p = .33$). MDA measurement was found on an average to be 63.7 in the control group, 22.7 in group 3 and 22.8 in group 4 ($p = 001$). *Conclusion*: Although tadalafil is superior to pentoxifylline, both drugs are considered to have positive effects.

Keywords: ischemic colitis; rat; pentoxifylline; phosphodiesterase-5; tadalafil; malondialdehyde

INTRODUCTION

Ischemic Colitis (IC) is the most common form of ischemic bowel disease, of which other subtypes include acute mesenteric ischemia and chronic mesenteric ischemia [1, 2]. The mortality rate is significantly higher in patients requiring surgery for treatment of this condition, The disease is reported to occur in 4.5–44 per 100.000 population. Ninety-eight percent of patients experience no complications when treated medically. However, more than half of the patients diagnosed with IC require surgery, and the mortality rate is 47% under these circumstances. IC occurs more frequently in women over 65 and in persons who have chronic obstructive pulmonary disease or irritable bowel syndrome [3–5].

Tadalafil is a phosphodiesterase-5 (PDE5) inhibitor drug that performs platelet aggregation inhibition and dilatation in the vascular smooth muscles. The drug is reported to have a higher therapeutic effect than sildenafil and vardenafil, both of which are from the same class of drugs [6–8]. Pentoxifylline is a

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derivative drug of methylxanthine with antithrombotic, anti-inflammatory, and hemorrhagic effects. In previous studies, it was reported to be effective in ischemic bowel models and ischemia/reperfusion models of small intestine, liver, and retina [9–12].

The aim of this study is to investigate the efficacy of tadalafil with pentoxifylline in rat models of IC.

MATERIAL-METHODS

The experimental protocol was established, and the Marmara University Experimental Animals Research and Implementation Center (DEHAMER) Ethics Committee approval, dated May 5, 2013 with protocol number March 31, 2013, was obtained prior to initiating the study. Thirty-two female rats, weighing 180–220 g, provided by DEHAMER were used for the study. Following a fasting period of 12 hr, the rats were weighed and placed under general anesthesia by using a combination of IM 3–5 mg/kg of chlorpromazine (Largactil® 25 mg/5 ml ampoules, Eczacıbaşı, Istanbul, Turkey) and 100 mg/kg of ketamine base (Ketalar® 500 mg vials; Pfizer, Istanbul, Turkey). The surgical procedures were performed in accordance with sterility standards. Following the administration of anesthesia, the abdominal region was cleaned with povidone iodine 7.5% liquid soap (Polyod®) and normal saline, shaved and stained with 1% povidone iodine, and the abdomen was then opened by making a 3-cm midline incision in accordance with aseptic principles. The left colon was identified in the rats in the sham group ($n = 8$). Three additional experiment groups including eight rats each were created. After the abdomen was opened, the left colon was identified and fixation was performed with 4/0 silk sutures by using the Griffin and Hagihara's method [13], and a 4-cm devascularized colon segment was created in the left colon. The abdomen was closed by continuous suturing using 3/0 silk sutures in all groups. The following substances were administered at the second hour of the postoperation period as the first day dose, and these doses were repeated for three days. The groups were developed as follows:

Group A (Sham group): Tap water was given through orogastric route following laparotomy ($n = 8$),

Group B (Control group): Tap water was given through orogastric route following laparotomy and induced IC ($n = 8$),

Group C (Pentoxifylline group): 50 mg/kg of SC pentoxifylline (Hemopene 100®, 100 mg/5 ml ampoules, İbrahim Ethem, Istanbul, Turkey) was administered following laparotomy and induced IC ($n = 8$),

Group D (Tadalafil group): 10 mg/kg of tadalafil (Lifta® 20 mg tablets, Abdi İbrahim, Istanbul, Turkey) was administered following laparotomy and induced IC ($n = 8$).

Following operation, the rats were monitored in cages housing two rats each in temperature ($22 \pm 2^{\circ}$C) and humidity ($50\% \pm 5\%$) controlled rooms with 12 hr light and dark cycles. Food and water requirements were fulfilled in line with their needs. The rats were observed postoperatively regarding the need for the use of analgesics. Analgesics were not used because there was no evidence of abnormalities such as abnormal general condition, insomnia, dehydration, lack of apettite, immobility, or abnormal movements. Therefore, we considered that analgesics were not necessary.

Seventy-two hours after the first operation, a thorough examination and blind evaluation was conducted on the rats that were chosen to undergo another operation. This second operation involved the same anesthesia as it was in the first operation and euthanasia was then followed by cervical dislocation and the opening of the abdomen through a U-shaped incision. Presence or absence of ascites, bowel dilatation, serosal change, and perforation was recorded by macroscopic evaluation. The devascularized colon segment was opened via excision and a measurement in mm^2 of the mucosal ischemic area was taken using graph paper, as described by Gomella et al. [14]. Macroscopic damage scoring, as described by Wallace et al. [15], was then performed. Accordingly, the scores were evaluated as follows: 0: Normal; 1: Local ischemia without ulceration; 2: Ulceration without hyperemia; 3: Ulceration (+), hyperemia in this area; 4: Ulceration (+), hyperemia in 2≤ areas; and 5: >2 cm ulcer in many areas.

After sampling for MDA assay, the colonic tissue was fixed in 10% formaldehyde, a paraffin block was generated and hematoxylen-eosin staining was done with 5 μm sections. A histopathological evaluation of the specimens was performed by a single pathologist under light microscope using blind evaluation based on Chiu scores [16]. Accordingly, the scores were evaluated as follows: 0: Normal mucosal villus; 1: Subepithelial Gruenhagen's space congestion; 2: Moderate separation of epithelial layer from lamina propria; 3: Massive epithelial separation of villus slices; stripping in several sites; 4: Villus separated from lamina propria and tense dilated capillaries; and 5: Digested and dispersed lamina propria; hemorrhage and ulceration.

The tissue sample collected for MDA was confirmed at -80°C and analyzed by high-performance liquid chromatography (HPLC). The result was calculated based on each gram of the colonic tissue (nmol/g).

Statistics

The data obtained from this study were analyzed with the help of SPSS 20 Package program. Two-group comparisons were performed by using Mann-Whitney *U* test, and for comparisons involving three or more groups, Kruskal-Wallis *H* and Bonferroni

TABLE 1 Macroscopic changes and ischemic area in the rats according to the groups

		A = Sham Group		B = Control Group		C = Pentoxifylline Group		D = Tadalafil Group		Statistics p and Paired Comparison
		n	%	n	%	n	%	n	%	
Acidic Fluid	No	8	100	0	0	8	100	7	87.5	A-B, B-C (.0001)
	Yes	0	0	8	100	0	0	1	12.5	B-D (.001)
Bowel Dilatation	No	8	100	0	0	6	75.0	7	87.5	A-B(.0001),B-C(.007)
	Yes	0	0	8	100	2	25.0	1	12.5	B-D (.001)
Serosal Change	No	8	100	0	0	8	100	7	87.5	A-B, B-C (.0001)
	Yes	0	0	8	100	0	0	1	12.5	B-D (.001)
Perforation	No	8	100	5	62.5	8	100	8	100	A-B, B-C, B-D (.201)
	Yes	0	0	3	37.5	0	0	0	0	
Adhesion	No	8	100	0	0	3	37.5	5	62.5	A-B(.0001),B-C(.201)
	Yes	0	0	8	100	5	62.5	3	37.5	B-D (.026)
Ischemic area (mm^2)		0.0		63.3		2.8		2.4		A-B, A-C, A-D, B-C, B-D (.0001)

corrected Kruskal-Wallis H test was employed. The correlation between the variables was examined by using Fisher's Exact Test. Significance level was considered 0.05 and 0.01 in paired comparisons (Bonferroni corrected Kruskal-Wallis H test).

RESULTS

No rat died throughout the study. The macroscopic findings observed in the first examination of the rats are shown in Table 1. As can be seen from the table, there are significant differences in terms of acidic fluid, bowel dilatation, serosal change, and intra-abdominal adhesion ($p < .01$), but no significant difference was noted in terms of perforation in any of the groups ($p = .201$). According to the ischemic area measurements performed using the Gomella technique, no ischemic area was detected in the sham group, while the average ischemic area was measured as 63.3 mm^2 in the control group, 2.8 mm^2 in the pentoxifylline group and 2.4 mm^2 in the tadalafil group. Significant differences were noted upon comparison of pentoxifylline and tadalafil between the sham and control groups ($p = .0001$).

Histopathological evaluation of the groups using Wallace Macroscopic Damage Scoring and Chiu Scoring are shown in Table 2. A significant difference was established between the sham group and the control and pentoxifylline groups ($p < .01$), but no significant difference was noted between the sham group and the tadalafil group ($p = .063$) in terms of Wallace score and Chiu classification. Similarly, a significant difference was observed between the control group and the pentoxifylline and tadalafil groups ($p < .01$), whereas no significant difference was established between the pentoxifylline group and the tadalafil group ($p = .33$).

Microscopic image samples functioning as the basis for Chiu scoring are shown in Figure 1.

The average value found in MDA measurements was 18.0 nmol/g in the sham group, 63.7 nmol/g in the control group, 22.7 nmol/g in the pentoxifylline group, and 22.8 nmol/g in the tadalafil group. Once again, significant differences were noted upon comparison of pentoxifylline and tadalafil in the sham and control groups ($p = .0001$).

TABLE 2 Wallace macroscopic damage scoring and chiu classification

		Wallace macroscopic damage scoring						Chiu scoring					
Cohort	Number of rats	0	1	2	3	4	5	0	1	2	3	4	5
A-Sham group	8	8	0	0	0	0	0	8	0	0	0	0	0
B-Control group	8	0	1	3	2	1	1	0	1	3	0	1	3
C-Pentoxifylline group	8	3	5	0	0	0	0	3	5	0	0	0	0
D-Tadalafil group	8	5	3	0	0	0	0	5	3	0	0	0	0
Paired comparisons (p value)		A-B = .0001, A-C = .009, A-D = .063; B-C VE B-D = .001; C-D = .333						A-B = .001, A-C = .009, A-D = .063; B-C VE B-D = .001; C-D = .333					

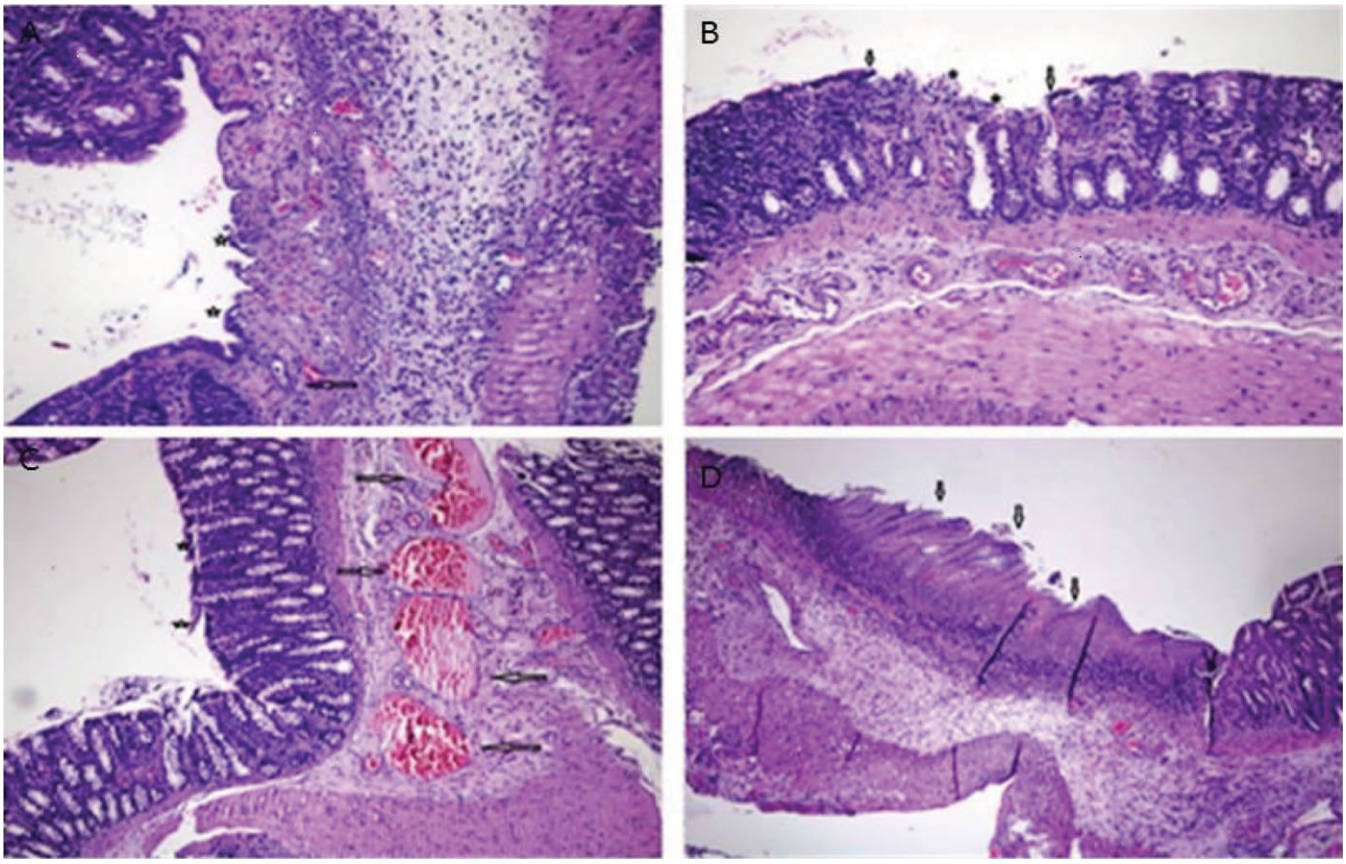

FIGURE 1 (A) The group designated as Grade 1 in the Chiu classification due to the presence of subepithelial Gruenhagen's space (marked by an asterisk) and capillary ejection (marked by an arrow) (H&Ex100). (B) The moderately separated epithelial (marked by an arrow) areas are accompanied by erosion on the surface (marked by an asterisk). Designated as Grade 2 in the Chiu classification (H&Ex200). (C) Massive epithelial separation (marked by an asterisk) areas and limited number of residual crypts (marked by an arrow) are distinguished in the deep mucosa. Designated as Grade 3 in the Chiu classification (H&Ex200). (D) Diffuse massive epithelial separation areas and ischemic changes (marked by an arrow) and presence of transmural necrosis. Designated as Grade 5 in the Chiu classification (H&Ex40).

DISCUSSION

Although IC occurs more frequently in the left colon, and specifically in the splenic flexura, its prevalence in the right colon has been reported to have increased gradually. The splenic flexura between both mesenteric blood flows is likely more affected by the intestinal diseases that result in low blood flow. The disease may vary in severity from mucosal superficial ischemia to transmural ischemia involving the intestinal wall and resulting structure [17]. There may also be differences in the clinical and macroscopic findings. In this particular study; the presence of acidic fluid, bowel dilatation, serosal change, and adhesions were more frequently seen in the control group, wherein the IC model was better demonstrated compared to the rats in the other groups ($p < .05$). However, perforation, which indicates severe colitis, was not frequent in any of the groups. Moreover, in accordance with the macroscopic damage scoring recommended by Wallace et al. [15] and previously used with confidence by investigators [18], serious changes were observed more often in the control group than in the other groups.

In our study, both pentoxifylline and tadalafil have been demonstrated to be effective according to the ischemic area measurement defined by Gomella [14] and MDA measurement, as defined by Uchiyama and Mihara [19], which is a significant indicator in IC and an indicator of lipid peroxidation level. The ischemic area was 63.3 mm^2 in the control group, but in the pentoxifylline and tadalafil groups, it was found to be only 2.8 and 2.4 mm^2, respectively. MDA was 63.7 nmol/g in the control group, whereas it was only 22.7 and 22.8 nmol/g in pentoxifylline and tadalafil groups, respectively ($p = .0001$). The efficacy of the drugs is considered to be the likely result of the common effects of these two drugs; namely, the inhibition of platelet aggregation, as well as the other well-known major effects associated with them. De Bon E et al. showed the effect of tadalafil on platelets and its useful effects on vascular endothelial ischemic damage [20]. In the study performed by JG Adams Jr et al. to investigate

the effect of pentoxifylline in the ischemic reperfusion model, the efficacy of pentoxifylline was proved by the inhibition of platelet-activating factor (PAF) [21]. Lloris Carsi JM et al. [9] also demonstrated a decrease in serum lactic acid dehydrogenase and tissue MDA levels after pentoxifylline administration in severe ischemia/reperfusion of small intestine; additionally they observed an increase in tumor necrosis factor-α, interleukin-1β and interleukin-6 levels. They also histologically noted a decrease in neutrophil infiltration in pentoxifylline group, but more severe necrosis and higher lysozyme levels in the control group.

The histopathological scoring described by Chiu et al. [16] has been widely accepted and used by many investigators. In addition, the correlation of Chiu scoring with macroscopic findings has been indicated by the researches performed [22]. Ischemia initially starts at mucosal layer and it extends to other layers, and Chiu scoring was found to be useful in describing the severity of ischemia. Considering the Chiu scoring from our study, it has been seen as remarkable that all scores in the pentoxifylline and tadalafil groups are Grade 0 and 1, and that, in contrast to the control group ($p = .001$), none of the rats had a score of Grade 3—4–5.

Determination of useful drugs in IC is important for the management of other mesenteric ischemic diseases. Acute mesenteric ischemia is particularly important in this group of conditions with a mortality rate of 59%–93% [23]. Accordingly, we believe that the effectiveness of pentoxifylline and tadalafil that we found in our study might also be applicable in other mesenteric ischemic conditions and/or ischemia/reperfusion models.

To conclude, pentoxifylline and tadalafil are considered to be effective in the rat model of IC.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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