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The Effect of Rectal Irrigation Using A Combination of Metronidazole and Normal Saline on Histopathological Grades of Hirschsprung-Associated Enterocolitis

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ABSTRACT

Background: Rectal irrigation is utilized both as a treatment and preventive approach for Hirschsprung-associated enterocolitis (HAEC). Normal saline is frequently employed for rectal irrigation, while metronidazole, a systemic antibiotic, is commonly used in managing HAEC. However, the use of metronidazole in rectal irrigation has yet to be explored. ***Objective:*** This study aims to evaluate whether rectal irrigation with a combination of normal saline and metronidazole results in a reduction in histopathological damage in HAEC compared to rectal irrigation with normal saline alone. ***Methods:*** This experimental study employed a randomized post-test-only control group design. A total of 22 Wistar rats with a Hirschsprung disease model were divided into two groups (11 rats per group). The experimental group received rectal irrigation with a combination of metronidazole and normal saline, while the control group received normal saline alone. After seven days, re-laparotomy was performed to collect intestinal samples, which were then analyzed for the histopathological severity of HAEC. ***Result:*** In the normal saline irrigation group, 5 rats showed mild infection, and 6 rats had moderate infection. In the group treated with the combination of metronidazole and normal saline, 7 rats exhibited mild infection, and 4 rats had moderate infection. Statistical analysis revealed no significant difference between the two groups ($p = 0.670$). ***Conclusion:*** The combination of metronidazole and normal saline in rectal irrigation does not significantly impact the histopathological grading of HAEC.

Keywords: Hirschsprung-associated enterocolitis (HAEC); rectal irrigation; metronidazole; normal saline; histopathological grading

INTRODUCTION

Hirschsprung disease is a congenital disorder that causes impaired intestinal motility.[1] Hirschsprung-associated enterocolitis (HAEC) is one of the major complications of Hirschsprung disease. Rectal irrigation, also known as rectal washout, is both a preventive and therapeutic intervention for HAEC. The incidence of HAEC is estimated to range from 6% to 60%, while the postoperative incidence is reported to be between 25% and 42%. The mortality rate associated with HAEC ranges from 5% to 50%, with a higher prevalence observed during the neonatal period prior to definitive surgical correction.[2]

In patients with HAEC, rectal irrigation must be performed promptly as a decompressive measure. A study conducted by Marty et al. (1995) demonstrated that routine postoperative rectal irrigation can reduce both the incidence and severity of enterocolitis in children with Hirschsprung disease. Normal saline is commonly used for rectal irrigation, while metronidazole, a systemic antibiotic, is frequently administered in the management of HAEC. However, the use of metronidazole as an agent in rectal irrigation has not yet been investigated.[3]

METHODS

This study was an experimental investigation employing a randomized post-test-only control group design. The experiment involved two randomly assigned groups, with a total of 22 rats included in the study. One group served as the treatment group, while the other functioned as the control group.

The treatment group underwent rectal irrigation using a combination of metronidazole at a dose of 7.5 mg/kg body weight and normal saline at 10 cc/kg body weight. The control group received rectal irrigation with 0.9% normal saline at a dose of 10 cc/kg body weight. Rectal irrigation was administered once daily for seven consecutive days.

Prior to rectal irrigation, the rats were first established as a Hirschsprung disease model using a 0.1% benzalkonium chloride (BAC) solution. Following completion of the rectal irrigation regimen, a relaparotomy was performed to obtain intestinal tissue samples. These samples were subsequently examined histopathologically to assess the severity of HAEC based on the histopathological grading system proposed by Teitelbaum.[4] In this study, grades 0, I, and II were classified as mild disease; grades III and IV as moderate disease; and grade V as severe disease.

RESULT

Clinical Features of the Large Intestine in a Wistar Rat Model of Hirschsprung Disease

Macroscopically, examination of the large intestine of rats treated with BAC on day seven revealed the presence of a transition zone. Colonic dilatation proximal to the transition zone was observed in one rat from the rectal irrigation group receiving a combination of metronidazole and normal saline, as well as in three rats from the rectal irrigation group receiving normal saline alone. Clinically, this dilatation may indicate the development of Hirschsprung-associated enterocolitis (HAEC).

FIGURE 1: (A) The black arrow indicates the transition zone between the dilated and non-dilated segments of the intestine. (B) Gross appearance of the large intestine in a rat exhibiting clinical features of HAEC. These findings were observed on day 7 following administration of 0.1% BAC solution.

Histopathological Changes in a Wistar Rat Model of Hirschsprung Disease

In this study, several colonic tissue samples were obtained from normal rats and from rats treated with 0.1% BAC followed by rectal irrigation. Intestinal samples from BAC-treated rats that clearly demonstrated a transition zone were selected for evaluation of ganglion cells.

In normal rats, ganglion cells were observed in both the myenteric and submucosal plexuses, with close interganglionic spacing. In contrast, rats with the Hirschsprung disease model exhibited wider spacing between ganglia within the myenteric plexus. To determine the histopathological severity of HAEC, intestinal tissue samples were taken from segments proximal to the transition zone.

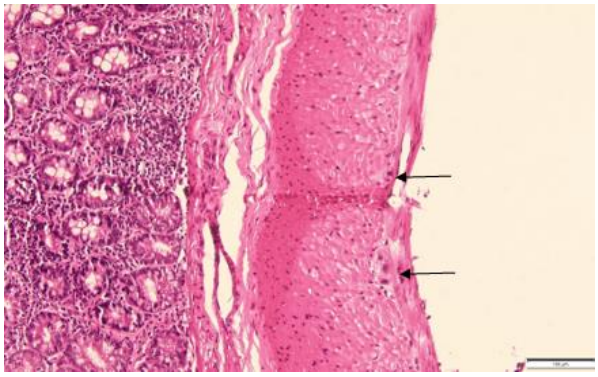

FIGURE 2: The black arrow indicates a ganglion in the myenteric plexus of the large intestine in a rat not treated with 0.1% BAC solution, observed at 200× magnification.

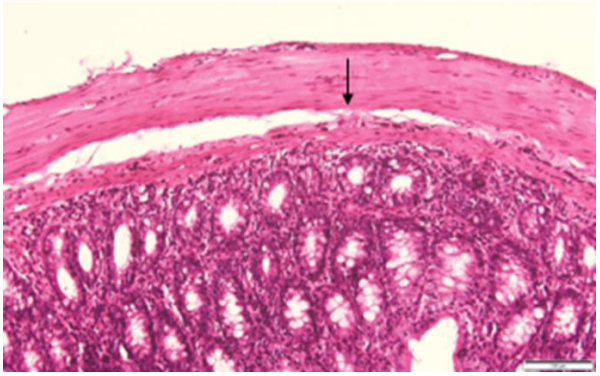

FIGURE 3: The black arrow indicates ganglion cells in the submucosal plexus of the large intestine in a rat not treated with 0.1% BAC solution, observed at 200× magnification.

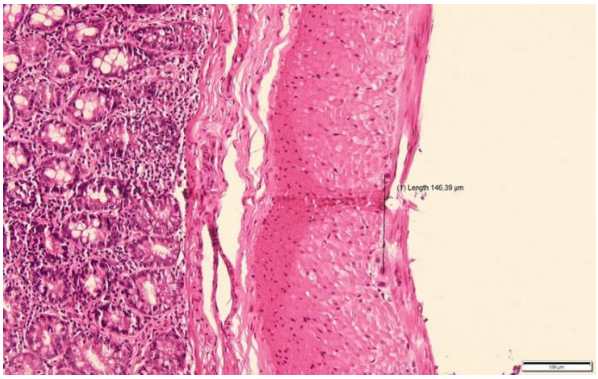

FIGURE 4: The distance between ganglion cells (146.39 µm) in the large intestine of a rat not treated with 0.1% BAC solution, observed at 200× magnification.

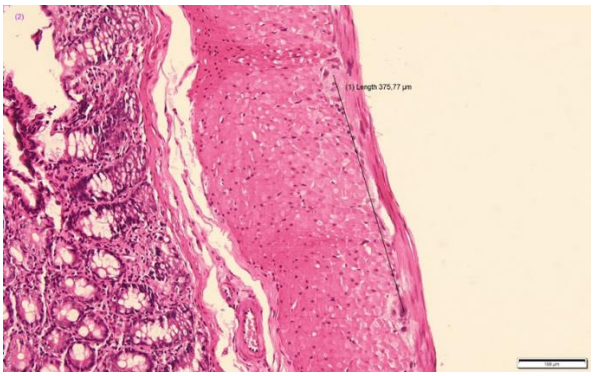

FIGURE 5: The distance between ganglia (375.77 µm) in the colon of rats not treated with 0.1% BAC solution, observed at 200× magnification.

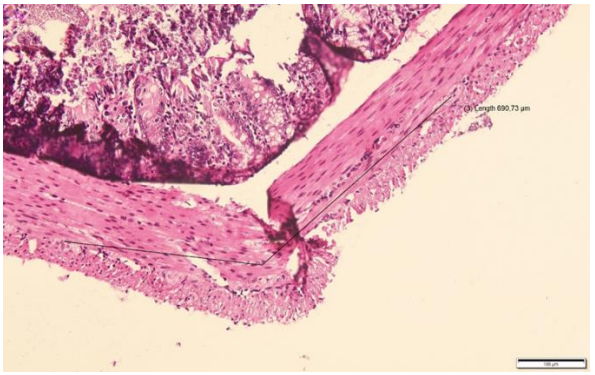

FIGURE 6: The distance between ganglia (690.73 µm) in the colon of rats treated with 0.1% BAC solution for 15 minutes, observed at 200× magnification.

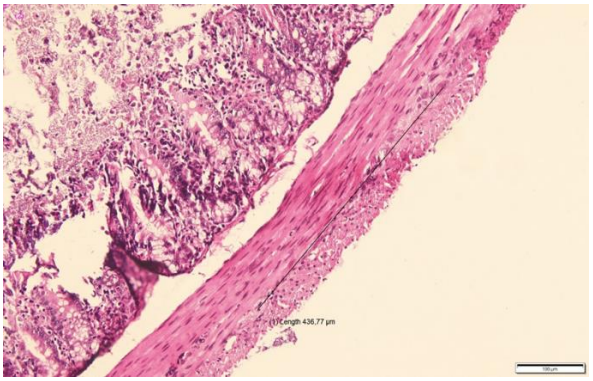

FIGURE 7: The distance between ganglia (436.77 µm) in the colon of rats treated with 0.1% BAC solution for 15 minutes, observed at 200× magnification.

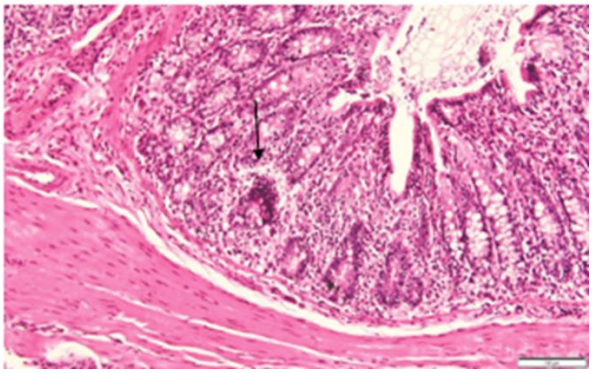

FIGURE 8: The black arrow indicates the presence of cryptitis, observed at 200× magnification.

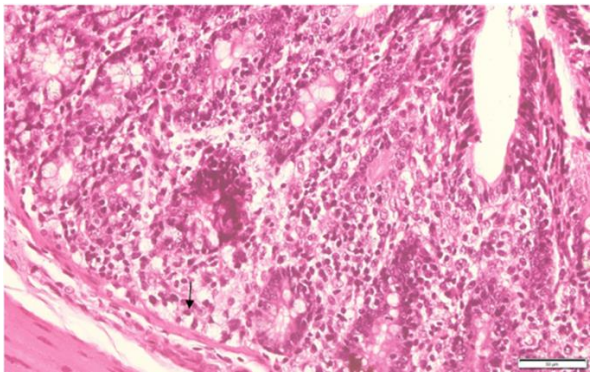

FIGURE 9: The black arrow indicates the presence of neutrophils, observed at 400× magnification.

Histopathological Severity of Hirschsprung-Associated Enterocolitis (HAEC)

TABLE 1: Histopathological Severity of Hirschsprung-Associated Enterocolitis (HAEC).

No	Normal Saline	Metronidazole + Normal Saline
1	Mild	Mild
2	Mild	Mild
3	Mild	Mild
4	Moderate	Mild
5	Mild	Mild
6	Moderate	Mild
7	Moderate	Moderate
8	Moderate	Moderate
9	Mild	Moderate
10	Moderate	Moderate
11	Moderate	Mild

The Effect of Rectal Irrigation with a Combination of Metronidazole and Normal Saline on the Histopathological Severity of HAEC

This study employed Fisher's Exact test to examine the relationship between rectal irrigation with a combination of metronidazole and normal saline and changes in the histopathological severity of HAEC. No significant difference ($p < 0.05$) was observed between rectal irrigation with the metronidazole - normal saline combination and rectal irrigation with normal saline alone.

TABLE 2: Effect of Rectal Irrigation with a Combination of Metronidazole and Normal Saline on the Histopathological Severity of HAEC.

Rectal Irrigation		Histopathological Severity of HAEC			Total	p-Value
		Mild	Moderate	Severe		
Normal Saline	Number	5	6	0	11	p = 0.670 (p > 0.05)
	Percentage	45.5 %	54.5 %	0	100 %	
Metronidazole + Normal Salin	Number	7	4	0	11	
	Percentage	63.6 %	36.4 %	0	100 %	

DISCUSSION

In this study, the distance between ganglia in the myenteric plexus of Hirschsprung's disease model rats treated with 0.1% BAC solution on day 7 was measured as 690.73 µm in one colon sample and 436.77 µm in another. Compared with normal colon tissue, the distance between ganglia in the myenteric plexus was greater in samples treated with 0.1% BAC than in the normal colon, which measured 146.39 µm and 375.77 µm. This finding aligns with Yu et al. (2016),

who reported that a 0.1% BAC treatment for 30 minutes may serve as a realistic model of Hirschsprung's disease rather than simple aganglionosis.[5]

Smith (1993) conducted a study using paraffin-embedded colon sections, 3 mm thick, stained with hematoxylin-eosin to examine ganglion counts in normal colon. Morphometric measurements were performed on autopsy colon tissues, and in 29 control cases, an average of seven nerve cells per mm of colon length was observed. Only one case showed hypoganglionosis, with fewer than two nerve cells per mm of large intestine.[6]

However, the observation that ganglion cells are better preserved in the submucosal plexus than in the myenteric plexus was not consistent with our findings, as no submucosal ganglia were detected in this study. This differs from Yu et al.'s findings, where submucosal ganglia were better preserved than myenteric plexus ganglia. This suggests that the effectiveness of BAC may depend on its permeability or activity range. Therefore, further studies are needed to elucidate the mechanism of BAC action and determine its effectiveness.

In this study, rats showing HAEC symptoms were evaluated subjectively based on observed signs, including reduced activity, decreased fecal output, and in some cases, loose stools. Other symptoms observed after 15 minutes of 0.1% BAC treatment included abdominal distension. Rectal irrigation with a combination of metronidazole and normal saline was administered to the treatment group, while the control group received rectal irrigation with normal saline alone. Treatments began on the first day after laparotomy. After seven days of treatment, the rats were reoperated, and colon samples were collected to assess HAEC severity based on histopathological abnormalities.

In the control group receiving normal saline irrigation, five rats exhibited mild infection, and six rats exhibited moderate infection. In the group receiving rectal irrigation with the metronidazole–normal saline combination, seven rats developed mild infection, and four rats developed moderate infection. No significant difference was observed between rectal irrigation with the metronidazole–normal saline combination and normal saline alone ($p = 0.670$).

Rectal irrigation is an important first-line therapy in HAEC management. It effectively relieves fecal stasis in patients with Hirschsprung's disease, thereby reducing bacterial overgrowth. Normal saline is a commonly used irrigation fluid. Currently, metronidazole remains a key therapeutic agent for HAEC, administered orally or parenterally.

Topical metronidazole has been tested in patients with Crohn's disease. A study by Stringer et al. (2005) reported that early trials with 10% metronidazole ointment were suboptimal. The 10% formulation aimed to deliver an effective local dose, with one inch of ointment containing approximately 70 mg of active drug. Absorption studies using liquid metronidazole showed that after a single topical application, serum concentrations were less than 5% of those achieved after a single 500 mg oral dose. No local or systemic adverse effects were observed from the topical formulation during the study.[7]

Topical metronidazole administration is considered a strategy to avoid systemic complications and achieve benefits such as improved local bioavailability and patient compliance. Metronidazole is highly active against gram-negative anaerobes (e.g., Bacteroides fragilis) and gram-positive anaerobes (e.g., Clostridium difficile). Its pharmacokinetic and pharmacodynamic properties are favorable, and it is available in oral, intravenous, vaginal, and topical formulations. After oral administration, metronidazole is well absorbed, with peak plasma concentrations occurring 1–2 hours post-dose.[8] According to the U.S. FDA, the intravenous loading dose is 15 mg/kg, followed six hours later by 7.5 mg/kg every six hours, yielding average peak (Cmax) and trough (Cmin) concentrations of 25 µg/mL and 18 µg/mL, respectively. The mean elimination half-life in healthy subjects is eight hours.

Metronidazole exhibits rapid bactericidal activity against anaerobes, with killing rates proportional to drug concentration. This concentration-dependent bactericidal effect has been demonstrated against Entamoeba histolytica and Trichomonas vaginalis. Additionally, metronidazole kills Bacteroides fragilis and Clostridium perfringens more rapidly than clindamycin and can cross the blood–brain barrier.[9]

In this study, intravenous metronidazole solution was used due to its availability compared with ointment. The dose applied was 7.5 mg/kg, equivalent to the intravenous regimen. The formulation and dose may influence metronidazole's effectiveness against anaerobic bacteria and protozoa, as well as its anti-inflammatory effects, which are concentration-dependent. The exact local absorption of metronidazole in this study could not be determined, as residual drug in the colon was not measured. During rectal irrigation with the metronidazole–saline combination, the irrigation fluid exited immediately with feces, making it impossible to determine the proportion of metronidazole acting on mucosal bacteria.

CONCLUSION

Based on the results and discussion of the study, the following conclusions can be drawn:

1. Clinically, the rats exhibited symptoms of HAEC, as subjectively observed from the manifestations that appeared.

2. Rectal irrigation using a combination of metronidazole and normal saline did not produce a statistically significant effect on changes in the histopathological severity of HAEC.

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