CLINICAL SCIENCE

EFFECTS OF THE HISTAMINE H2 RECEPTOR ANTAGONIST FAMOTIDINE ON THE HEALING OF COLONIC ANASTOMOSIS IN RATS

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BACKGROUND: Failure of anastomotic healing is one of the major complications in colorectal surgery. Because histamine plays an important role in immune and inflammatory reactions, we demonstrate the effects of famotidine on the healing of colonic anastomosis in rats.

METHODS: Twenty-eight Sprague-Dawley rats were used in the study. Excision and end-to-end anastomosis was performed in the distal colon of the rat. The Famotidine Group received 2 mg/kg/day famotidine; the Control Group received the same amount of saline. Bursting pressure of anastomoses and hydroxyproline content of perianastomotic tissues were evaluated on the third and seventh days following surgery.

RESULTS: Bursting pressures and hydroxyproline contents for the Famotidine Group were significantly lower than the equivalent parameters for the Control Group on both the third and seventh days post-surgery.

CONCLUSIONS: According to our findings, famotidine exerts detrimental effects on the anastomotic bursting pressure and hydroxyproline content of perianastomotic tissues in the colon of rats.

KEYWORDS: Colonic anastomosis; Anastomotic healing; Famotidine; Histamine.

INTRODUCTION

Anastomotic leakage is a serious complication in colorectal surgery.[1-3] Leakage rates vary greatly and are associated with 0 to 30% of all cases. Clinically apparent leakage rates are between 2.1 and 14.9%.[1,2] Failure of anastomotic healing is associated with increased duration of hospital stay, morbidity and mortality.[2-4] At least one-third of deaths following colorectal surgery are attributed to anastomotic leakage, although the reduction in the incidence of anastomotic dehiscence is due to contributing factors such as advances in perioperative care, bowel preparation, and surgical techniques.[3]

Healing of colon anastomoses involves a complex interaction of peptide growth factors and collagen turnover through the phases of inflammation, fibroplasias, and maturation.[5-7]

Histamine plays an important role in immune reactions, in the coagulation cascade, and in various related inflammatory reactions. Histamine H2 receptors accelerate cell proliferation, thereby impacting both lymphocyte and immune system response.[8,9]

Acid suppressive drugs (H2 receptor antagonists and proton pump inhibitors) are used for the prevention and treatment of stress-related mucosal diseases and other acid-related disorders.[10]

In the context of these histamine-induced effects, we decided to examine the effects of famotidine, an H2 receptor antagonist, on the healing of the anastomosis of the colon.

METHODS

Twenty-eight Sprague-Dawley male rats weighing 230-255 g were used in this experiment. A biostatistician

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was consulted to determine the appropriate number of rats per group. The animals had free access to water and were fed standard rodent chow throughout the experiment. Rats were housed for one week prior to surgical intervention. The Ethics Committee of Fatih University Medical School approved the experiment.

All animals were anesthetized by subcutaneous injection of 30 mL/kg ketamine (Ketalar, Eczacıbaşı, Türkiye). The abdomen was shaved and prepared using povidone-iodine and a sterile dressing. A laparotomy was performed with a 2 cm lower midline incision. An end-to-end anastomosis was performed after excision of a 0.5 cm segment of the distal colon, 3 cm proximal to the peritoneal reflection. Ten to twelve interrupted 6/0 polypropylene (Prolene, Ethicon) inverting sutures were used in the anastomosis. The abdominal fascia was closed with 3/0 continuous polyglactine (Polysorb, Tyco) sutures, and the skin was closed with 4/0 polyglactine (Polysorb, Tyco) in a subcuticular manner.

The animals were randomly assigned to two groups. The Famotidine Group (14 rats) received 2 mg/kg/day (0.5 mL/kg/day) famotidine (Nevofam-L amp, Mustafa Nevzat), while the Control Group (14 rats) received the same amount of saline subcutaneously. This dose of famotidine is almost equivalent to double the maximal dose used clinically. The justification for this dose selection was to more clearly elicit the possible effects of famotidine. Following surgery, the rats were allowed free access to water and resumed standard feeding.

Seven rats from each group were randomly selected to assess the bursting pressure and hydroxyproline content of anastomosis on the third day following surgery. The remaining seven rats from each group underwent the same procedure on the seventh day following surgery.

After anesthesia was maintained with 30 mL/kg Ketamine, the abdomen was opened, and the whole colon was resected. Great attention was paid to preserve the adhesions. Wide resections were performed when necessary. The proximal colon was attached to a continuous pressure monitoring system with a transducer (Petaş, KMA365B) and supplied with air (1 mL/minute) with an air pump. The distal colon was occluded. The entire colon was immersed in saline, and the air pump was activated. The maximum pressure before bubbles were seen was recorded as the bursting pressure for each rat. After the bursting pressure was determined, the peri-anastomotic region was cleared. A 1-cm colon segment (0.5 cm from each side of the anastomosis) was resected for hydroxyproline determination and stored at -40°C. Hydroxyproline content determinations of the samples were performed as described by Jamall et al.[11]

SPSS for Windows® 10.0 was used for the statistical analysis. Differences between groups in terms of bursting pressure and hydroxyproline content were assessed using a Mann-Whitney *U* test. The statistical significance was set to $p<0.05$.

RESULTS

The effects of famotidine on healing of a colonic anastomosis were examined in rats. All rats survived the experiment, and all anastomoses were intact on both the third and seventh days post surgery.

Recorded bursting pressures for the Famotidine Group were significantly lower than those for the Control Group on both the third and seventh days post surgery (Table 1). The mean hydroxyproline content was significantly lower for the Famotidine Group than for the Control Group (Table 2).

Table 1 - Mean bursting pressures of the Control and Famotidine Groups

Day	Group	Number of rats	Bursting pressure (Mean±SD mmHg)	p-value
3	Control	7	131.43±53.31	<0.05
	Famotidine	7	79.57±21.11	
7	Control	7	209.43±18.14	<0.05
	Famotidine	7	188.29±14.26	

Table 2 - Mean hydroxyproline contents of the Control and Famotidine Groups

Day	Group	Number of rats	Hydroxyproline Content (Mean±SD µg/mg tissue)	p value
3	Control	7	2.92±0.25	<0.05
	Famotidine	7	2.34±0.63	
7	Control	7	4.63±0.41	<0.05
	Famotidine	7	2.65±0.28	

DISCUSSION

Wound healing is a complex cascade of overlapping events that depends on a number of cellular mechanisms and signaling pathways. It requires the coordinated completion of a variety of cellular activities that include phagocytosis, chemotaxis, migration, proliferation, adhesion and differentiation. The end result is synthesis and cross-linking of collagen and remodeling of the connective tissue.[6,12,13] The process of anastomotic healing is similar to observations in the skin.[14] The inflammatory phase is essential for healing, with hemostasis preceding inflammation.[15] Any factor that disrupts one or more steps in the healing process will likely

result in impaired anastomotic healing.[16] Anastomotic dehiscence is a serious complication that causes significant morbidity and mortality in colorectal surgery.[2,4,17]

Bursting pressure and hydroxyproline determinations are measures that offer insight into the anastomosis healing process.[2,18-20] Bursting pressure reveals the mechanical parameters of a colonic anastomosis and reflects growing anastomotic strength. Biochemical parameters of anastomotic healing are reflected by the collagen content in perianastomotic tissues, as determined by hydroxyproline content.[19] In our experiments, we measured the bursting pressure of the anastomosis and the hydroxyproline levels of the perianastomotic tissue to determine healing rates.

Histamine significantly influences healing.[21] Histamine is one of the regulators of mesenteric blood flow, leading to vasodilatation and increasing the regional blood flow in the mesentery[22]. Histamine offers homeostatic control of the circulation under both normal and pathologic conditions and causes vasodilatation of the intestine.[23] H2 receptor antagonists inhibit the vasodilatory effects of histamine.[22] However, blood supply is one of the determinants of anastomotic healing and is of paramount importance.[24,25]

Histamine also takes part in the regulation of proliferation and angiogenesis and may play an important role in the growth of both normal and malignant tissue.[8] The blood supply is dependent on the formation of new blood vessels in the anastomosis. [25]

An initial consequence of injury is the exposure of collagen in the vascular sub-endothelium. Platelets attach to collagen, secrete their granule constituents, and form aggregates.[26] Histamine H2 receptor antagonists reduce platelet aggregation.[27]

Histamine participates in the regulation of immune reactions, in the coagulation cascade and in inflammatory responses and the formation of proinflammatory mediators.[8,28] Histamine H2 receptors accelerate cell proliferation and affect the immune system. H2 receptor antagonists reverse the histamine-induced increases in IL-4, IL-5, and interferon-γ levels.[8] IL-4 plays a role in wound fibroplasia, and interferon-γ has effects on fibroblast proliferation, both of which are components of wound healing.[15,29]

Cimetidine, an H2 receptor antagonist, effectively inhibits inflammation-generated increases in nitric oxide concentrations.[28] It is known that nitric oxide plays role in phagocytosis and antimicrobial function and wound healing.[15]

Some studies have found that pretreatment with H1 antagonists improves survival in the context of shock, while H2 antagonists exacerbate mortality in low flow scenarios.[22]

Very few studies have explored H2 blockers in the context of colorectal anastomosis. Ranitidine has been shown to have no effect on experimental anastomotic strength, but it did result in a lower incidence of septic complications in an animal study. We cannot directly compare the results of this study to our conclusions, as hydroxyproline levels were not reported and the bursting pressure was determined in a different manner.[9] In our study, famotidine exerted a significant influence on anastomotic strength and decreased the collagen content of anastomoses in rats.

In conclusion, our study demonstrates that famotidine reduces the bursting pressure of colonic anastomosis and may negatively impact the collagen content of perianastomotic tissue. This is likely a result of impairment of the positive influences of histamine on healing. Further studies are needed to explain the mechanisms of action of famotidine in the context of anastomoses and to determine whether these findings are applicable to humans.

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