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Effects of Ghrelin on the Oxidative Stress and Healing of the Colonic Anastomosis in Rats

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ABSTRACT

Background: Anastomotic leakage is the deadliest complication of colonic procedures. Ghrelin is an orexigenic hormone with potent actions on growth hormone release and functions in the processes of growth, tissue inflammation, repair, and oxidative stress. We evaluated the hypothesis that the exogenous administration of ghrelin causes beneficial effects on the healing of colonic anastomosis.

Materials and methods: Sixty-four male Wistar rats were randomly assigned to eight subgroups receiving postoperative intraperitoneal administration of ghrelin (23 μg/kg/d) or saline after a colonic anastomosis. The anastomotic tissue was evaluated on the third, seventh, and 14th postoperative days. Anastomotic bursting pressure, histological parameters, hydroxyproline content, and tissue oxidative stress markers were compared.

Results: There was a significant increase in the mean anastomotic bursting pressure in the ghrelin subgroup on the seventh postoperative day ($P = 0.035$). Histological evaluation demonstrated a significant difference in the neutrophilic infiltrate ($P = 0.035$) on the third and 14th d and in apoptosis ($P = 0.004$), granulation tissue ($P = 0.011$) and peritoneal inflammation ($P = 0.014$) on the 14th postoperative day. There was a statistically significant increase in the hydroxyproline content in the ghrelin subgroup on the 14th postoperative day ($P = 0.043$). There were significant differences in the nitrite tissue levels ($P = 0.021$) on day 3 and in reactive oxygen species ($P = 0.012$) on day 14.

Conclusions: The administration of ghrelin had beneficial anti-inflammatory and antioxidant effects, increasing the resistance of the anastomosis and the hydroxyproline tissue content in the postoperative period.

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Introduction

Colorectal surgery has incorporated important technical advances and scientific knowledge in the last several decades, including staplers, antibiotics, bowel preparation, and laparoscopic access, all of which have improved the safety and efficacy of this procedure.[1,2] Nevertheless, colorectal procedures are still associated with important postoperative complications. Anastomotic leakage is the most severe complication and has an incidence between 2% and 16%. It is associated with higher rates of morbidity and mortality, higher hospital costs, and worse oncological results.[3-5]

Ghrelin was originally identified, purified, and characterized by Kojima *et al.*[6] in 1999 from rat stomach. It subsequently received notoriety as the first known endogenous ligand of the GH-secretagogue receptor (GHS-R). Ghrelin is a potent GH-releasing and appetite-stimulating peptide consisting of 28 amino acids, in which serine 3 is modified by a fatty acid (n-octanoic acid) in the activated form.[7]

It has been demonstrated in experimental studies that the intracerebral, intraperitoneal, or subcutaneous administration of ghrelin avoids damage to the gastric mucosa and accelerates healing in ethanol ischemia/reperfusion injury and in injuries due to alendronate or hydrochloric acid. Ghrelin increases the release of local nitric oxide (NO)[8] and the serum levels of tumor necrosis factor-α (TNF-α) and nuclear factor kappa B (NF-κB), which stimulate proinflammatory tissue cytokines.[9] In addition, ghrelin has decreased myeloperoxidase and malonyldialdehyde activity[10] and decreased cell apoptosis, cytochrome P, and caspase 3 release, which are associated with oxidative stress and healing delays.[11]

In the bowel, it has been proven that ghrelin administered intravenously or intracerebroventricularly has important postoperative protective effect against the damage induced by ischemia and reperfusion in rats. Ghrelin induces a reduction in the release of inflammatory cytokines (TNF-α and IL-6) and neutrophilic infiltrate by decreasing myeloperoxidase activity.[12] Sen *et al.*[13] found a significant decrease in NF-κB levels, recently described these findings and showed the inhibition of glutathione oxidase depletion in the ileal segments after ischemia/reperfusion injury, suggesting an antioxidant protective effect.

It has also been recently demonstrated that the administration of ghrelin in rats subjected to acetic acid–induced colitis increases blood flow and local DNA synthesis in the inflamed colonic mucosa, exhibiting potent anti-inflammatory actions, promoting a significant reduction in the levels of TNF-α, IL-1β and myeloperoxidase activity, and accelerating the process of cell regeneration.[14,15]

These data demonstrated that ghrelin influences and improves repair and intestinal healing, acting as an anti-inflammatory or antioxidant agent. In the present study, we demonstrated that the administration of ghrelin in the postoperative period after colonic anastomosis could attenuate inflammation, apoptosis, and oxidative stress and improve the anastomotic bursting pressure in rats.

Materials and methods

Animals and surgical procedures

Sixty-four male Wistar rats (*Rattus norvegicus*) from the Central Animal Facility at the Federal University of Santa Catarina (UFSC) were used in this research, which was previously approved by Ethics Committee on Animal Use of UFSC, under case PP0915/146–2014. The animals weighed between 300 and 350 g and were between 160 and 180 d old. They were acclimated for 7 d at the Laboratory of Experimental Surgery and Operative Technique of the UFSC while receiving food and water ad libitum in standardized conditions. The room was maintained at a constant temperature of 23 ± 2°C, with light and dark cycles alternating every 12 h and humidity from 50% to 60%. All procedures were performed in accordance with the Brazilian guidelines for animals in research.

The procedures were performed with the animals under general anesthesia by using an intramuscular solution of ketamine (9 mg/100 g body weight) and thiazine (1.25 mg/100 g body weight). A 2-cm middle laparotomy was performed under aseptic conditions. The sigmoid colon was identified and sectioned with straight scissors 2 cm above the peritoneal reflection. Then, an end-to-end anastomosis was performed with eight separate stitches of 6-0 polypropylene (Ethicon, Somerville, NJ) in the extramucosal layer. Abdominal wall closure was performed in two planes with continuous 4-0 nylon sutures (Ethicon, Somerville, NJ).

Reoperations were performed under the same conditions. The prior anastomosis was identified, and the sigmoid colon containing the anastomosis segment was extracted for analysis.

Groups

The animals were randomized into two groups based on the postoperative administration of ghrelin (G) or saline (S). Each group was distributed into four subgroups of eight rats ($n = 8$), which were named according to the time of the reoperation and euthanasia in the postoperative period: sham saline (SS), sham ghrelin (SG), saline and 3 d (S3), ghrelin and 3 d (G3), saline and 7 d (S7), ghrelin and 7 d (G7), saline and 14 d (S14), and ghrelin and 14 d (G14) (Fig. 1).

In the saline group, the animals received 1 mL of saline solution; in the ghrelin group, the animals received 23 μg/kg/d of rat ghrelin (Tocris, Bioscience, Bristol, UK) dissolved in 1 mL of saline solution. In both groups, the administration was made by daily intraperitoneal (IP) injection during the postoperative period with randomization.

Anastomotic bursting pressure measurements

A 2-cm sigmoid segment containing the anastomosis centrally was identified and maintained in the abdominal cavity. One end was closed with 2-0 silk suture, and the other end was introduced into a 6-Fr polyvinyl catheter occluded with 2-0 silk thread. The catheter was connected in a "Y" configuration system to a continuous infusion pump (Samtronic, São