10-Review

Ghrelin and gastrointestinal wound healing. A new perspective for colorectal surgery[1]

Humberto Fenner Lyra Junior[I], Igor Kunze Rodrigues[II], Leonardo de Lucca Schiavon[III], Armando José D`Acâmpora[IV]

[I]PhD, Assistant Professor, Department of Surgery, Universitary Hospital, Universidade Federal de Santa Catarina (UFSC), Florianopolis-SC, Brazil. Conception and design of the study; acquisition, analysis and interpretation of data; manuscript preparation and writing.
[II]MSc, Department of Surgery, Universitary Hospital, UFSC, Florianopolis-SC, Brazil. Conception and design of the study, acquisition of data.
[III]PhD, Associate Professor, Internal Medicine, Universitary Hospital, UFSC, Florianopolis-SC, Brazil. Analysis and interpretation of data, critical revision, final approval.
[IV]PhD, Associate Professor, Department of Surgery, Universitary Hospital, UFSC, Florianopolis-SC, Brazil. Critical revision, final approval.

■ Introduction

Healing of intestinal anastomosis remains a challenge for surgeons, mainly after colon and rectal resections. Anastomotic leakage is the most feared and potentially fatal complication after colorectal surgery because usually results in high morbidity and mortality, increase length of stay, medical costs and is associated with poorer long-term oncological results[1-5]. The prevalence of anastomotic leakage has been reported to range from 1 to 26%[1,2,6-11]. Multiple factors are involved in healing process of colorectal anastomosis, including technical-related factors and patients-specific characteristics. Over the last few years, molecular aspects of wound healing process have been described and great interest is being given in the role of hormones in the reparative process. The aim of the present review is to provide a comprehensive analysis of the role of ghrelin`s therapy in the gastrointestinal anastomotic healing mainly in colorectal procedures.

■ Gastrointestinal healing process

Gastrointestinal healing process is a multifactorial and complex process that depends on a several local and systemic factors and have three distinct phases that begins immediately after the tissue damage[12-14]. The complete healing process results in an optimal balance of all phases that are described below:

- Inflammation or “lag” phase: It starts immediately after the tissue injury, until third postoperative day. The fibrin-based clot is created by the efflux of platelets and an increase of permeability promotes the migration of neutrophils resulting in a hemostasis and epithelial restitution;
- Proliferation phase: Proliferation process begins in the wounded site with fibroblasts in the fourth postoperative day

that becomes the major local cell type. Several growth factors acting in this period manly platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and transforming growth factor (TGF-β). At this time the matrix established in the inflammatory phase is replaced with granulation tissue that promotes angiogenesis and high levels of oxygen and nutrients to satisfactory healing process;

- Remodeling phase: Generally, after fourteen days the reorganization process occurs with decrease of macrophages and fibroblasts, thickening of collagen fibers and wound contraction leading to maturation state.

Although the basic healing process is similar in the skin and in the gastrointestinal tract (GIT), there are some specific differences, which directly influence tissue repair. For example, there are three collagen subtypes (1, 3, 5) in the GI and only two (1, 3) in the skin. The collagen is produced by fibroblasts and smooth muscle cells in GI and only by fibroblasts in the skin. Anaerobic and aerobic bacteria are present in GI lumen whereas only aerobic in the skin. The collagenase activity is greater in the GI tract wound repair especially in the first three days and the anastomotic strength is weaker in this period. This condition does not occur in the skin repair. Anastomotic ischemia and tension-free suture are important and crucial factors in the healing process of GI tract and poor tissue perfusion strongly contributes for anastomotic dehiscence[14] (Table 1).

Table 1 - Comparison between GI tract and skin healing process

	GI tract	Skin
Collagen (subtypes)	1,3,5	1,3
Producing collagen cells	Fibroblasts / Smooth muscle	Fibroblasts
Wound environment	Aerobic / Anaerobic	Aerobic
Collagenase activity	High	Low
Tissue perfusion status	Significant	Not significant

■ Major factors affecting healing process in colorectal anastomosis

The reasons for anastomotic breakdown are unclear and several risk factors and clinical conditions have been reported in the genesis of colorectal anastomotic dehiscence[6,11]. Generally, the risks can be stratified as patient-specific, technical-specific and low anastomosis risk factors.

Obesity is an independent risk factor for anastomotic leak (AL) and increase the risk of leakage up to 2.7 times when compared with non-obese patients[6,15]; this association has many hypotheses like tissue stricture and healing defect, increase of intraabdominal pressure and microcirculation damage. Nutritional status is another important factor. Many studies have demonstrated the correlation of low serum protein levels and AL showing a decrease of anastomotic complications with preoperative nutritional improvement[6,8,10].

Smoking and alcohol abuse are conditions that have also been associated with AL because they cause microvascular disease and ischemia and consequent anastomotic healing failure[16]. Also, preoperative long–term steroid use strongly increase the rate of leakage of colorectal procedures when

compared with non-steroid use[17]. Many studies have demonstrated that radiotherapy is another independent factor and is involved in anastomotic healing defect mainly in rectal resections probably by inducing an inflammatory response and local ischemia[6,16]. Colorectal surgery series have demonstrated others patient-specific conditions involved in colonic anastomotic healing process like ASA status, perioperative transfusion and cardiovascular diseases that directly affects anastomosis formation and complete healing[5,6].

The most important technical-specific factors involved in healing process are mechanical and manual suturing, bowel preparation, proximal defunctioning stoma and anastomotic level. Although evidence are scarce, results comparing hand sewn versus stapled anastomosis found lower leak rates in the mechanical procedure in the majority of the series[18-20]. This benefit is more evident in low an ultra-low colorectal anastomosis than in the colonic or ileocolic anastomosis. Diverting stoma also is a controversial condition because it does not act directly in the healing process; it only prevents major septic complications by diverting fecal stream and sometimes delays the fistula diagnosis in the postoperative period. Nowadays this procedure should be considered in patients who underwent low rectal anastomosis (below 6cm from anal verge), neoadjuvant radiotherapy and critically ill patients[10,11,16].

Finally, mechanical bowel preparation remains a controversial perioperative condition in the processes of anastomotic dehiscence. The primary goals of mechanical cleansing are: to reduce the colonic bacterial load, decrease surgical site infection and improves the handling of the bowel leading to a better anastomotic healing[21-26]. A recent experimental study demonstrated a negative impact of bowel preparation on cellular proliferation and intracellular mechanisms and consequently on anastomotic healing[27]. Further researches are necessary to clarify and to set the best preoperative method for elective colorectal procedures.

Strategies for healing improvement of colorectal anastomosis

Therapeutic interventions for optimizing a healing anastomotic process have advanced in the last decades. Studies have demonstrated many options and parameters to achieve the best results in intestinal healing. Animal models, surgical techniques, pharmaceutical interventions and different segments of GI tract have been tested and demonstrated different results[14,28].

Øines *et al*.[28] in a recent meta-analysis identified seven therapeutic agents for improve the colonic anastomotic healing and that could be explored further. They stratified the agents in four categories: immunomodulators, miscellaneous, proteinase inhibitors and growth hormone (GH) factors.

In immunomodulators class, only ileoprost and tacrolimus demonstrated significant results, improving the anastomotic healing and increasing an early bursting pressure when compared with controls. None of the other tested substances showed benefits of anastomosis healing.

In experimental series, proteinase inhibitors agents have consistently demonstrated an improvement of anastomotic strength in the early postoperative days and are candidates for further studies. A single clinical trial showed that intravenous administration of aprotinin have reduced the anastomotic leakage in low colorectal anastomosis when compared with placebo group[29].

Miscellaneous category is represented by hyperbaric oxygen therapy (HBOT),

hypothermia, antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), hormones and GH factors as described below:

Oliveira *et al*.[30,31] reported a detrimental effect of perioperative hypothermia on the colonic anastomotic healing in animal models with decrease of inflammatory cytokines (IL-1, IL- 6, IL-10) and growth factors (IGF-1, VEGF) in the seventh and fourteenth postoperative days.

HBOT has been demonstrated as an important tool to improve the anastomotic healing in the ischemic and inflamed mucosa in colonic anastomosis by anti-infection effect and reducing an ischemia-reperfusion injury[32-34]. The effect appears not to be limited to the direct influence of high oxygen levels, but also related to an increment of local production of inflammatory cytokines and consequently the inflammatory response.

NSAIDs have been associated with a high-risk of anastomotic leak after gastrointestinal and colorectal surgery. Some authors recommend that the NSAIDs should be abandoned after primary colonic anastomosis because of the increase of leak rate that has been consistently demonstrated in experimental and clinical studies[35-40]. This find is due to an effect on collagen metabolism leading to weakened tissue around the anastomosis and on the risk of thrombosis formation leading to a decrease of anastomotic blood flow and ischemic damage.

Experimental studies demonstrated controversial effects of perioperative antibiotics administration in the gastrointestinal wound healing process. There is no consistent results to recommend antibiotics for prevent anastomotic leakage[28].

The hormones and GH factors therapy (erythropoietin, GH an IGF-1) have been studied and demonstrated important and promising results in gastrointestinal healing. Sorg *et al*.[41] reviewed the erythropoietin effects in stages of reparative process and conclude that it increases the local levels of vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS) and consequently protects against ischemia damage particularly after surgical procedures and may lead to clinical benefits. Exogenous GH and IGF-1 increase the collagen levels in anastomotic area, but can presents local collateral effects as increasing the granulation tissue, leading a healing deficit and consequently an anastomotic leak[28].

Recently, the exogenous administration ghrelin, an orexigenic peptide, was evaluated in the healing of colon anastomosis of rats resulting in a significant burst pressure improvement when compared with control group without collateral effects[42]. These new approaches require further studies to determinate pathophysiology mechanisms and clinical applicability.

Ghrelin

Ghrelin was originally identified, purified and characterized by Kojima *et al*.[43] from rat stomach. Received notoriety, because is the first known endogenous ligand of GH-secretagogues receptor (GHS-R). It is a potent GH-releasing and appetite-stimulating peptide consisting of 28-amino acids, in which serine 3 position is modified by a fatty acid (n-octanioic acid) in the activated form[44,45].

The major forms of circulating ghrelin are n-octanoyl-modified and des-acyl ghrelin regulated by food intake and fasting conditions. The nonacylated form is inactive and circulates in amounts greater than acylated form[46]. Experimental and clinical studies have reported ghrelin-producing cells and ghrelin mRNA expression in different tissues and organs as kidney, large bowel, rectum, small bowel, thyroid, human placenta, brain, adrenal, ovary,

testis, heart and pancreas[47-51].

The two primary physiological functions of active form are release GH and increase the food intake. Additional effects also have been reported on cardiovascular system, gastrointestinal physiology, metabolism of glucose, lipids and bone[44-46,52].

Three pathways control ghrelin-stimulated GH release: direct effect on pituitary cells, direct effect from the hypothalamus and indirect effect by vagus nerve pathway signaling which increases noradrenaline in the arcuate nucleus of the hypothalamus leading a central control of feeding behavior. This is an indirect pathway control because of the peptide do not pass the blood-brain barrier in the high levels[46,53]. Date *et al*.[54] have demonstrated that vagotomy abolish fast-induced elevation of ghrelin plasma levels.

Effects of ghrelin in the gut

Protective effects of ghrelin have been demonstrated in different segments of the gut and for distinct conditions (Table 2).

Table 2 - Protective ghrelin effects in the gut.

	Main mechanisms	Clinical / Physiological consequences
Vascular effects	↑ local NO release	Reduction of granulation tissue
	↓ tissue congestion	Improvement of healing remodeling phase
	↑ vascular permeability	Attenuation of organ injury after gut I/R
Immunomodulatory properties	↓ cellular infiltration	Healing acceleration of inflamed mucosa
	↓ apoptosis	Reduction of neutrophil infiltration
	↑ GH	Decreasing of intestinal barrier dysfunction
	↓ IL-1 β	Stimulation of collagen synthesis
	↑ IGF-1	Improvement of anastomotic integrity
	↓ TNF α	
	↑ PGE -2	
Oxidative stress process	↓ MDA	Antioxidant effects
	↓ Myeloperoxidase activity	Reduction of inflammatory response
	↓ NF-ķB	Reduction of ROS tissue levels
	↑ Catalase	Protective effect against membrane damage
	↑ Superoxido dismutase	Acceleration of healing process
	↑ GSH peroxidase	
Functional effects	↑ gastric emptying	Improvement of postoperative recovery
	↑ intestinal motility	Early postoperative feeding
	↑ food intake (appetite)	Promotion of body weight gain

NO: nitric oxide; IL: interleucine; TNF: tumor necrosis factor; MDA: malonydialdehyde; ROS: reactive oxygen species; OS: oxidative stress; GH: growth hormne; IGF: insulin growth factor; PG: Prostaglandin; I/R: ischemia/reperfusion injury: NF-kB: nuclear factor kappa B; GSH: Glutathione peroxidase

In gastric mucosa ghrelin administration reduces the damage evoked by ethanol, alendronate, oxidative stress (OS), gastric resection, acetic acid, diabetic inflammation and ischemia-reperfusion (I/R) state[55]. El Eter *et al*.[56] have reported an antioxidant effect of

ghrelin in the gut by increasing nitric oxide (NO) release, leading to reduce ulceration, tissue congestion and increase vascular permeability and cellular infiltration.

In animal models, ghrelin administration has promoted acceleration on the healing of gastric and duodenal ulcers by releasing endogenous GH and IGF-1 and leading an increasing of mucosa blood flow and local cell proliferation rate[57]. Warzecha *et al*.[58] also have demonstrated an improvement of healing process in cisteamine-induced duodenal ulcers assessing reduction of mucosal OS and inflammatory response and reduction of mucosal OS and inflammatory response Ercan *et al*.[59] have reported decrease of apoptosis and OS in sodium metabisulfite induced gastric injury, suggesting that ghrelin treatment attenuates gastric injuries and could improve approaches against gastric mucosal injury.

Exogenous ghrelin prevents decreasing of the antioxidant glutathione in ileal I/R injury and attenuates excessive inflammation and intestinal damage in vagotomized rats after I/R gut-induced injury. It promotes activation of the cholinergic pathway by intracerebroventricular injection and results in inhibition of NF-kB activation in ileal mucosa and an increasing of antioxidative enzymes release (eg., superoxido dismutase, catalase and GSH peroxidase)[60,61]. Intravenous ghrelin administration also induces inflammatory cytokines release, ameliorates intestinal barrier dysfunction and reduces neutrophil infiltration in I/R-induced intestinal injury in animal models[62]. Intraperitoneal administration of ghrelin has increased gastric acid secretion and protected against water immersion and restraint stress injury in experimental study[63]. Adami *et al*.[64] also have found protective effects in gastric lesions induced by intragastric administration of HCL in rats, this effect was abolished by prior administration of GHS-receptors antagonists and inhibition of histamine release.

Ghrelin proprieties in colonic healing has also been reported in different studies on colitis and inflammatory bowel diseases (IBD) showing several therapeutic effects and results depends on the type of colitis etiology and damage time[65].

Konturek *et al*.[66], in an experimental study, have assessed an anti-inflammatory effect of exogenous ghrelin administration in induced colitis due to the increasing of nitric oxide release by stimulation of COX-2-derived PGE2 and consequently improvement of mucosal healing.

Therapeutic effect of ghrelin in acute colitis was associated with reduced lipid peroxidation and TH1-induced inflammatory response, but these effects are less effective than in chronic colitis[67]. Cheng *et al*.[68] have demonstrated a prevention of the breakdown of intestinal barrier function in dextran sulfate sodium-induced colitis in rats with reduction of transepithelial electrical resistance and tight junction expression, and bolstered tight junction structural integrity and regulated cytokine secretion[69].

In IBD, serum ghrelin and obestatin levels are significantly higher in patients with active disease than in patients with remission, and colonic mucosal mRNA expression of ghrelin was also significantly higher in patients with active disease than in patients in remission mainly in Ulcerative Colitis (UC) suggesting as activity marker in UC[70].

Maduzia *et al*.[71] have reported protective effects of ghrelin in the large bowel in acetic acid-induced colitis with a reducing mucosal concentration of IL-1beta and malondialdehyde concentration, as well as decreased mucosal activity of myeloperoxidase improving anti-oxidative effects and healing process.

Matuszyk *et al*.[72] have assessed anti-inflammatory and healing effect in acetic

acid-induced colitis in rats and the relationship between ghrelin and colonic healing, demonstrating faster regeneration of the colonic wall and reduction in colonic levels of IL-1beta, TNF-alpha and myeloperoxidase leading to curative effect on injured colonic tissue.

All these findings suggests that exogenous ghrelin protects and exerts beneficial anti-inflammatory and anti-oxidant effects in colonic mucosa, resulting in better intestinal mucosal healing and could be applied to ameliorate the results in colorectal surgery.

Ghrelin in abdominal and colorectal surgery

Experimental and clinical studies have been reported the influence of exogenous ghrelin in anastomotic healing, clinical recovery and postoperative ileus after abdominal procedures involving stomach, esophagus and colon[73,74].

Adachi *et al*.[75] have showed in a randomized controlled phase II trial with 21 patients, that ghrelin postoperative administration leads a decrease of body weight loss in ghrelin group after total gastrectomy when compared with placebo group (P=0.044), because of the orexigenic and prokinetic effects. Takigushi *et al*.[76] also have demonstrated that postoperative administration of synthetic ghrelin in gastrectomized patients improve oral food intake (P=0.039) and body weight gain (P=0.037) after one year of procedure when compared with control group in a recent clinical trial.

Popescu *et al*.[77] in a phase 2b study with 236 patients have reported accelerated motility recovery of the upper and lower gastrointestinal tract in the group that received an intravenous infusion of ghrelin agonist TZP-101 after partial colectomies, with less adverse events within 72 hours of postoperative period when compared to placebo. Takata *et al*.[78,79] have reported less pulmonary complications and decreasing of C-reactive protein and IL-6 levels and consequently reduction of systemic inflammatory response syndrome duration in the postoperative period in patients undergoing esophagectomy that received continuous infusion of ghrelin when compared with saline group (P=0.0065).

The postoperative antifibrotic effects of ghrelin were also demonstrated by Bianchi *et al*.[80] in growth hormone secretagogue receptor-knockout (GHSR KO) mice, that received postoperative intraperitoneal administration of ghrelin, and had significantly reduction of postoperative adhesion formation ($P < 0.001$) when compared with control group. The improvement of postsurgical outcomes were confirmed by measuring collagen I protein levels via Western blot analysis and demonstrating that this effect is mediated by the GHSR-1a receptor[80].

Recently, Ceran *et al*.[42] in an experimental study have reported another promising clinical effect of ghrelin. They have assessed a beneficial effect of postoperative ghrelin administration on the healing of colonic anastomoses in rats when compared with controls. They have showed an increasing in the bursting pressure and hidroxyproline levels in the ghrelin group on the seventh postoperative day. This results was associated with increase of fibroblasts efflux, local collagen synthesis and consequently an increasing of the burst pressure. All these findings evidences a potential benefit for abdominal procedures and new investigations should be performed to elucidate the real role of this peptide in the healing process, especially in colonic anastomosis.

In Table 3, summarizes the main clinical and experimental studies investigating the relationship between exogenous ghrelin administration and postoperative protective effects.

Table 3 - Proposed postoperative protective effects of ghrelin in GI procedures.

Reference	Year	Subjects	Procedure	Main Effects
Adachi *et al*.[75]	2010	Humans	Total Gastrectomy	Decrease of body weight loss, increase of food intake and postoperative appetite.
Popescu *et al*. [77]	2010	Humans	Partial Colectomy	Reduction of first bowel movements time, faster recovery of gastrointestinal function and early hospital discharge.
Ceran *et al*.[42]	2013	Animals	Partial Colectomy	Increase of BP and hydroxyproline tissue level, lower intraperitoneal adhesions formation.
Takata *et al*.[78]	2015	Humans	Esophagectomy	Shorter SIRS duration, lower CRP and IL-6 levels, decrease of inflammatory response and lower pulmonary complications.
Bianchi *et al*.[80]	2016	Animals	Laparotomy	Reduction of peritoneal adhesions and fibrotic response, anti-adhesion effect.
Takiguchi *et al*.[76]	2016	Humans	Gastrectomy	Increasing of postoperative food intake, appetite and body weight gain.

BP: Bursting pressure; SIRS: Systemic inflammatory response syndrome; CRP: C-reactive protein; IL: Interleukin.

■ Conclusions

Data available in the literature shows numerous proprieties and action mechanisms of ghrelin, not only in feeding or in GH releasing, but also in remodeling cellular process that could be essential in the majority of the abdominal procedures. Advances have recently been made in our understanding of the relationship between ghrelin and the pathophysiology of wound healing process, oxidative state and inflammatory response. These beneficial interactions in the anastomotic healing process and protective tissue effects represent opportunities for the evaluation of new therapeutic strategies focused on the gastrointestinal surgery.

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Correspondence:
Humberto Fenner Lyra Junior
Departamento de Cirurgia – CCS, Campus Universitário
Rua Professora Maria Flora Pausewang, s/n
88036-800 Florianópolis - Santa Catarina Brasil
Tel.: (55 48)99963-7506
humbertolyrajr@gmail.com

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