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Ghrelin ameliorates adhesions in a postsurgical mouse model

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ABSTRACT

Background: Peritoneal adhesion formation is a well-recognized consequence of abdominal and pelvic surgery, causing infertility, chronic pelvic pain, and intestinal obstruction. We hypothesized that ghrelin, a 28-amino acid peptide predominantly found in the stomach, plays an important role in preventing postoperative surgical adhesions. The purpose of this study was to develop a new surgical peritoneal adhesion model to define the role that ghrelin plays in wound healing and adhesion formation.

Materials and methods: C57BL/6 wild-type mice ($n = 40$) and growth hormone secretagogue receptor-knockout (GHSR KO) mice ($n = 20$) underwent a midline laparotomy to establish a peritoneal adhesion model characterized by the combination of two different techniques: ischemic peritoneal buttons and cecal multiple abrasion. All mice received intraperitoneal injections with ghrelin (0.16 mg/kg) or saline twice daily for 20 d after surgery. Peritoneal ischemic buttons were harvested to determine protein expression of collagen (Masson trichrome, picrosirius red stain, and Western blot).

Results: The novel mouse model demonstrated consistent and easily reproducible formation of intra-abdominal adhesions. Ghrelin administration significantly reduced postoperative adhesion formation ($P < 0.001$) in wild-type mice. The antifibrotic effect of ghrelin in wild-type mice was confirmed by measuring collagen I protein levels via Western blot analysis. The anti-adhesion effect of ghrelin seen in wild-type mice was not detected in GHSR KO mice demonstrating that this effect is mediated by the GHSR-1a receptor.

Conclusions: Ghrelin administration may improve surgical outcome by reducing peritoneal adhesion formation and fibrotic response in a mouse model.

1. Introduction

Intraperitoneal adhesion formation is a well-recognized consequence of abdominal and pelvic surgery. Postoperative intra-abdominal and pelvic adhesions are a leading cause of infertility, chronic pelvic pain, and intestinal obstruction [1]. The incidence of adhesion-related readmissions according to the surgical procedure ranges between 0.1% and 24%, with the highest incidences occurring after pelvic surgery [2]. In 2011, adhesiolysis was reported to be responsible for 351,777 hospitalizations, 976,332 d of inpatient care, and $2.3 billion in hospitalization and surgeon expenditures [3,4]. Beyond the

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economic impact, these postsurgical adhesions are responsible for hospital readmissions and delays in patient recovery. The presence of adhesions may increase the risk of inadvertent visceral injuries and lengthen the operation time during subsequent laparotomies [5,6]. The risk of needing repeat abdominal surgery is relatively high and is expected to increase in the Western world with the increase in life expectancy and developments in surgical technique [7,8].

The pathophysiology of adhesions originates from an inflammatory reaction stimulated by tissue trauma with increased vessel permeability, extravasation of immune cells, and deposition of fibrin. In ischemic conditions, such as after surgery, physiological fibrinolysis is insufficient leading to the formation of fibrin bridges between the areas of traumatized tissue and unaffected tissue. Subsequent organization of fibrin matrix with neovascularization and invasion of fibroblasts and myofibroblasts under the influence of specific growth factors ultimately causes the formation of fibrous adhesions [9].

Numerous interventions have been developed to reduce or prevent postsurgical adhesion formation, including fibrinolytic agents [10], antibiotics, anti-inflammatory agents [11], surgical lysis [12], anticoagulants [13], and chemical and physical barriers [14,15]. Unfortunately, none of these approaches has proven to be reliable and effective at preventing the formation of postoperative adhesions and subsequent complications. Currently, the only adhesion prevention strategy approved by Food and Drug Administration is Seprafilm (Genzyme Biosurgery Corp, Cambridge, MA) [16,17]. Although Seprafilm, a bioresorbable physical barrier composed of hyaluronic acid and carboxymethylcellulose, reduces adhesion formation by preventing apposition of injured tissue where placed; there are significant limitations to its use including difficulties with application and their effectiveness appears to be limited to the site of application [18,19]. The anti-adhesion mechanism of the Seprafilm barrier is thought to act *in vivo* as a physical barrier to separate peritoneal tissue surfaces during the early phases of wound healing [20].

Based on data from preliminary studies, we hypothesized that acylated (AG) ghrelin, a 28-amino acid gastric peptide, would reduce postoperative adhesion formation. Ghrelin was first isolated and characterized from the rat stomach and also identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR) [21]. The endocrine activities of ghrelin are mediated by the GHSR, and although primarily studied as a gastric hormone, it is also expressed in many other tissues where it works in a paracrine manner [22]. Ghrelin circulates in an AG and des-acylated form (dAG) [21]. The presence of the acyl side chain attached to the serine 3 residue of the ghrelin peptide is required for binding to the receptor GHSR-a1 [21]. The majority of ghrelin (>90%) circulates in human plasma as dAG form, although the exact ratio of circulating AG to dAG varies depending on metabolic status [23]. This effect is due to the shorter half-life of AG compared to the dAG. The AG plasma level rapidly disappears from circulation owing to binding to the GHSR-1a in the systemic tissue [24] or deacylation by circulating butyrylcholinesterase or carboxylesterase [25]. Previous studies suggest that most AG circulates bound to lipoproteins, whereas dAG flows as a free peptide [26]. Two GHSRs subtypes, GHSR-1a and GHSR-1b, have been identified [21]. GHSR-1a mediates ghrelin's effects on growth hormone secretion; while the function of the GHSR-1b is still unclear [27]. GHSR-1a is a G protein–coupled receptor expressed in the pituitary gland to mediate growth hormone release and in the hypothalamus to stimulate food intake and appetite [21,28]. The GHSR-1a receptor is also expressed in several peripheral organs, such as the bowel, pancreas, liver, heart, lungs, kidneys, and testes, facilitating the multiple paracrine, autocrine, and endocrine actions of ghrelin [28].

Ghrelin exercises a large range of biological functions, including anti-inflammatory effects [29] and cardioprotective effects [30]. It also alleviates anorexia, cachexia, chronic diseases [31–33], and renal damage [34]. Moreover, ghrelin improves renal function after ischemia–reperfusion injury and is shown to be involved in the healing of colonic anastomoses [35]. Recently, ghrelin was suggested to possess antifibrotic properties [34,36] and based on these observations, we hypothesized that this endogenous ligand may play an important role in reducing postoperative adhesion formation. This study developed a new experimental mouse model for the consistent induction of postoperative adhesions, which allows for analysis and evaluation of the effectiveness of ghrelin in reducing the formation of intraperitoneal adhesions. We demonstrated the capability of ghrelin administration to reduce postsurgical adhesion formation in a GHSR-dependent manner.

2. Materials and methods

2.1. Chemicals

Rat-lyophilized AG ghrelin was obtained from Tocris Bioscience (Bristol, United Kingdom). Ghrelin was dissolved in sterile saline (0.9% sodium chloride; Baxter Healthcare Corporation, Deerfield, IL) before injection.

2.2. Animals

GHSR KO mice, C57BL/6 mice with a deletion of the $GHSR^{-/-}$, were developed at Baylor College of Medicine. $GHSR^{-/-}$ mice were backcrossed at least 10 generations to C57/BL6 mice to create an isogenic line. Male C57BL/6 wild-type mice were purchased from Charles River Laboratories (Wilmington, MA). A total of 40 male wild-type mice and 20 male GHSR KO, aged 50–55 d and weighing between 19 and 21 g, were used in the study. All animals were allowed free access to Purina Rodent Chow 5001 (Farmer's Exchange, Framingham, MA) and filtered tap water *ad libitum*. Mice were housed in the Brown University Animal Care Facility and maintained in a temperature-(25°C–28°C) and humidity-controlled environment (30%–70%) with a 12-h alternating dark–light cycle. Body weights of the experimental animals were recorded immediately before the surgical procedure. All investigations were conducted in accordance with Guide for the Care and Use of Laboratory Animals and were approved by the Brown University Institutional Animal Care and Use Committee.