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Teduglutide effects on gene regulation of fibrogenesis on an animal model of intestinal anastomosis

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ABSTRACT

Background: Teduglutide is an enterotrophic analog of glucagon-like peptide 2 approved for the rehabilitation of short-bowel syndrome. This study aims to analyze the effects of teduglutide administration on the gene regulation of fibrogenesis during the intestinal anastomotic healing on an animal model.

Methods: Wistar rats ($n = 62$) were assigned into four groups: "Ileal Resection and Anastomosis" or "Laparotomy," each one subdivided into "Postoperative Teduglutide Administration" or "No Treatment," and sacrificed at the third or at the seventh days, with ileal sample harvesting. Gene expression of matrix components and remodeling factors (matrix metalloproteinases [*Mmp*] and tissue inhibitors of metalloproteinases [*Timp*]) and growth factors was studied by real-time polymerase chain reaction. Net collagen deposition was assessed through the *Collagen*-to-*Mmp*-to-*Timp* ratio of fold change of relative gene expression.

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Collagen
Matrix metalloproteinases
Tissue inhibitors of metalloproteinases

Results: Gene expression profiles revealed a balance toward net degradation of collagen at the third day of the intestinal anastomotic healing. Teduglutide appeared to be associated with an overall accumulation of collagen at the third day of the anastomotic repair, attributable to the upregulation of *Collagen type 1 alpha 1*, *Collagen type 3 alpha 1*, and *Collagen type 4 alpha 1*, *Timp1*, and *Timp2* and downregulation of *Mmp13* and to a net degradation of collagen at the seventh day, derived from repression of *Collagen type 3 alpha 1*, *Collagen type 5 alpha 1* and *Timp1* expression.

Conclusions: Teduglutide appeared to be associated with a favorable influence on fibrogenesis at the third day of the intestinal anastomotic repair and to a trend to fibrolysis at the seventh day.

Introduction

Despite recent progresses in operative technique and perioperative care, failure of intestinal anastomotic healing remains one of the most serious postoperative complications,[1] often associated with high morbidity and mortality rates, prolonged hospitalization, poor functional results, significant impact on quality of life, and considerable economic costs.[2]

Intestinal anastomotic repair is a well-orchestrated process, regulated by cytokines and growth factors, that evolves in three overlapping phases: inflammatory, proliferative (including re-epithelialization, fibroplasia, and angiogenesis), and remodeling.[1,3]

Extracellular matrix is a dynamic network of macromolecules with particular physical, biochemical, and biomechanical properties[4] that confers structural stability to the tissues, supports organ function and repair, and constitutes a key element of the epithelial stem cell niche.[4,5]

Control of the extracellular matrix turnover during the anastomotic wound healing process depends on a rigorous balance between synthesis and degradation and involves interactions between extracellular matrix components, proteases (matrix metalloproteinases [Mmps]), and proteases inhibitors (tissue inhibitors of matrix metalloproteinases [Timps]).[6,7] Failure to maintain the homeostasis of the extracellular matrix, with disturbances of the balance between synthesis and degradation of its components, seems to constitute an important factor in the pathogenesis of the intestinal anastomotic failure (dehiscence or stenosis).[6,8-10] Higher expression of MMP1, MMP2, and MMP9 in biopsies obtained perioperatively from patients' colon was associated with an increased rate of subsequent anastomotic leakage.[8] In addition, the treatment with Mmp inhibitors has been shown to improve the mechanical strength in animal models of colonic anastomotic healing.[6]

Several experimental studies have been undertaken on the role of adjuvants of intestinal anastomotic healing, including growth factors, in standard or in high-risk contexts. Nevertheless, until now, no clear evidence was obtained to support the implementation of any of those strategies in routine clinical practice.[3,11]

Teduglutide is a long-acting dipeptidyl peptidase IV–resistant analog of glucagon-like peptide 2 (GLP2),[12] an enterotrophic growth factor produced in enteroendocrine L cells,[13] recently approved by the United States Food and Drug Administration for the pharmacologic rehabilitation of adult patients with short bowel syndrome.[12]

Response of the intestinal fibrogenesis to teduglutide administration in the perioperative context of intestinal anastomosis is not well understood.

This study aims to analyze the effects of teduglutide short-term administration on the gene regulation of fibrogenesis during the intestinal anastomotic healing on an animal model.

Methods

Study protocol

Adult male Wistar *albinus* rats were randomly assigned into four groups: "Ileal Resection and Anastomosis" ("Res") or "Laparotomy" ("Lap"), each one subdivided into "Postoperative Teduglutide Administration" ("Ted+") or "No Treatment" ("Ted−"). Evaluation was performed at the moments of the operation and sacrifice, at the third or the seventh postoperative days (eight subgroups), with recovering of ileal segments (except during isolated laparotomy). All the laboratorial analyses were performed in a blinded manner for the experimental groups.

Study was approved by the Ethics Committee of the Faculty of Medicine, University of Coimbra, Coimbra, Portugal, and undertaken according institutional and national animals' protection guidelines.

Animals

Animals weighting 250-300 g were acclimatized to the laboratory environment for 5 d before experimental study, kept in temperature (22 ± 1°C) and humidity (50 ± 10%) controlled ventilated cages with light–dark cycles of 12 h, and maintained on water and standard rodent diet *ad libitum*.

Surgical interventions

All the operative procedures were performed by the same surgeon after a period of 2-h fasting (water was never restricted) and determination of the preoperative weight, with clean surgical technique and under anesthesia with an intraperitoneal injection of ketamine hydrochloride (75 mg/kg; Pfizer Inc, NY) and chlorpromazine (3 mg/kg; Laboratórios Vitória, Amadora, Portugal).