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Doxycycline administration improves fascial interface in hernia repair

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ABSTRACT

Background: Despite improvements in ventral hernia repair techniques, their recurrence rates are unacceptably high. Increased levels of matrix metalloproteinases (MMPs) and reduced collagen-1 to -3 ratios are implicated in incisional hernia formation. We have recently shown doxycycline treatment for 4 wk after hernia repair reduced MMP levels, significantly increased collagen-1 to -3 ratios, and increased tensile strength of repaired interface fascia. However, this increase was not statistically significant. In this study, we extended treatment duration to determine whether this would impact the tensile strength of the repaired interface fascia.

Materials and methods: Thirty-two male Sprague–Dawley rats underwent incision hernia creation and subsequent repair with polypropylene mesh. The animals received either saline ($n = 16$) or doxycycline ($n = 16$) beginning from 1 day before hernia repair until the end of survival time of 6 wk ($n = 16$) or 12 wk ($n = 16$). Tissue samples were investigated for MMPs and collagen subtypes using Western blot procedures, and tensiometric analysis was performed.

Results: At both 6 and 12 wk after hernia repair, the tensiometric strength of doxycycline-treated mesh to fascia interface (MFI) tissue showed a statistically significant increase when compared with untreated control MFI. In both groups, collagen-1, -2, and -3 ratios were remarkably increased in doxycycline-treated MFI. At 6 wk, the doxycycline-treated MFI group showed a significant decrease in MMP-2, an increase in MMP-3, and no change in MMP-9. At 12 wk, MMP-9 showed a remarkable reduction, whereas MMP-2 and -3 protein levels increased in the doxycycline-treated MFI group.

Conclusions: Doxycycline administration results in significantly improved strength of repaired fascial interface tissue along with a remarkable increase in collagen-1, -2, and -3 ratios.

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1. Introduction

Ventral hernias represent one of the most prominent surgical problems in the United States with incisional hernias affecting more than 11% of patients after a major abdominal wall surgery [1]. Among patients who undergo a hernia repair, there is up to a 23% recurrence rate of this condition [2]. Despite advancements in prosthetic materials technology

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and surgical procedures, this unacceptably high rate of recurrence is a persisting problem. The high incidence of incisional hernia and a higher rate of recurrence are often linked to an underlying connective tissue disorder [3]. Collagen is the predominant structural protein of the extracellular matrix (ECM) that mostly contains collagen 1 and 3 along with other collagen types. For a structurally stronger abdominal wall, there needs to be a higher ratio of more mature collagen 1-to-less mature collagen 3 [3]. This ratio is affected by the activities of a class of endogenous proteases called matrix metalloproteinases (MMPs) with individual MMPs affecting specific ECM components [4]. Tetracyclines inhibit MMPs independent of their antibacterial actions [5]. Doxycycline is a semisynthetic chemically modified tetracycline compound widely used to treat infections caused by Gram-negative and Gram-positive microorganisms. Doxycycline is a broad spectrum MMP inhibitor that also is known to scavenge toxic reactive oxygen species (ROS), thus preventing the conversion of pro-MMPs to active MMPs [6]. Our preliminary studies have shown that treatment with doxycycline led to a marked reduction of MMP-2 and MMP-9 in the repaired interfacial tissue of rats with repaired hernias after surgery while there was no change observed at earlier time points [7]. At this time point, there was also an increase in tensile strength (TS) of the repaired fascial tissue; however, it did not reach statistical significance. Therefore, we conducted another study with longer duration of doxycycline treatment after surgery to see if that would lead to a statistically significant difference between control and treatment groups.

2. Materials and methods

2.1. Animal methodology (experimental design)

The animal experimental protocol was previously approved by the Institutional Animal Care and Use Committee of the University of Kentucky, and the complete procedure was conducted according to their guidelines. Thirty-two male Sprague–Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN), acclimatized for 5 d, and randomly assigned to one of two groups: group 1, treatment group receiving doxycycline 1 d before incisional hernia repair ($n = 16$) and group 2, control group receiving saline oral gavage 1 d before hernia repair ($n = 16$). Before both hernia creation and repair surgeries, animals underwent a general anesthesia with inhaled 4% isoflurane, and the abdominal wall was clipped and prepped with 70% chlorhexidine. After each surgery, pain control was achieved by subcutaneous buprenorphine injection (0.03 mg/100 gm).

2.2. Animal procedures

The protocol used for incisional hernia creation was detailed previously [7]. Each rat had undergone an initial hernia creation procedure. After prepping the ventral midline area, a 1-cm transverse incision was made at the level of the xiphoid. A full-thickness skin flap was then created at the avascular prefascial plane above the linea alba. Under direct visualization, the linea alba was cut for 4 cm to make a full-thickness laparotomy. The skin was closed in two layers. The inner subcutaneous layer was closed using a 4.0 polyglycolic acid interrupted suture, whereas the outer layer was closed using wound clips or an interrupted 4.0 nylon suture. Triple antibiotic ointment was placed on the incision, and the animals were observed until ambulant. The rats were monitored after the surgery for possible complications for 4 wk. At 27 d after the first surgery, the treated animal group started receiving daily oral gavage of 30 mg/kg doxycycline until euthanasia, whereas the control animals received normal saline. The 30 mg/kg dose was selected as this dose was previously shown to elicit a maximum MMP-2 inhibition in 2-kidney one-clip hypertensive rats [8].

At the sixth and 12th wk time points after mesh implantation surgery, eight animals from both the control and treated groups were euthanized by CO_2 asphyxiation, and their abdominal wall tissue harvested. Small pieces of tissue from both repaired and normal area were collected; one part of it was snap frozen in liquid nitrogen, and part of the remaining tissue was processed for paraffin sections.

2.3. Western blotting

MMP-2, -3, and -9, and collagen 1 and 3 antibodies for Western blotting were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA), and the blotting was performed using a previously described protocol [7]. Briefly, lysis buffer (1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 0.1 mg/mL phenylmethylsulfonyl fluoride, 2 mg/mL aprotinin, 2 mg/mL leupeptin, 2 mg/mL Pepstatin A, and 1× phosphate-buffered saline) was added to frozen abdominal wall tissue (one part tissue to four parts lysis buffer) and homogenized using an Ultra-Turrax homogenizer (Tekmar Co, Cincinnati, OH). Homogenates were centrifuged at 10,000*g* for 20 min, and the supernatants were again centrifuged at 100,000*g* for 1 h. Supernatants were collected, aliquoted, and stored at −80°C. One aliquot was used for a protein assay using the bicinchoninic acid protein assay protocol (Pierce Protein Biology Products, Rockford, IL). The samples were denatured by boiling for 5 min with 2× gel loading buffer (17.3% glycerol, 1.25 M β-mercaptoethanol, 5.2% sodium dodecyl sulfate, 0.22MTris, pH 6.8, 1×10^2 mg bromophenol blue). Thirty microgram of protein from each sample was electrophoresed (4% stacking gel and 8.5% separating gel at 175 V for 1 h) and then electroblotted onto polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc, Hercules, CA) at 100 V for 1 h. Membranes were then incubated for 1 h in a blocking buffer (5% fat-free dry instant powdered milk, 1 mM Tris-base, 15 mM sodium chloride, and 0.05% Tween-20) at room temperature with shaking. The primary and secondary antibodies were diluted in blocking buffer. They were then incubated with the membrane while shaking for 1 h, starting with the primary antibody and followed by the secondary antibody at room temperature. The membranes were then washed with three changes of wash buffer (1 mM Tris-base, 15 mM sodium chloride, and 0.05% Tween-20) after the primary and secondary antibody incubations. A Super Signal chemiluminescent substrate kit (Pierce, Rockford, IL) was used to detect antibodies bound to the membrane, and the images were analyzed by ImageJ software (NIH, Bethesda, MD).

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