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Topical vanadate enhances the repair of median laparotomy incisions

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ARTICLE INFO

Article history:
Received 11 May 2016
Received in revised form
1 August 2016
Accepted 24 August 2016
Available online 31 August 2016

Keywords:
Laparotomy
Hernia
Wound healing
Scar
Vanadate

ABSTRACT

Background: There are over two million laparotomies performed in the United States each year with an incisional hernia rate between 2% and 11%. A total of 100,000 ventral hernia repairs are undertaken each year with recurrences as high as 50%.

Materials and methods: Full thickness midline fascia incisions from the xiphoid to the pubic symphysis were made in rats. The fascia and/or muscular layer was sutured closed and a gel with 300 μM of sodium orthovanadate or saline was placed over the suture line with the skin closed over it. On day 10, 1-cm strips from the superior, middle, and inferior regions of the abdominal wall were tested for breaking strength and processed for histology.

Results: The mean wound breaking strength of vanadate-treated wounds was 18.6 ± 2.7 N compared with 9.4 ± 3.6 N for controls ($P < 0.0001$). Similar quantities of granulation tissue were deposited in treated and control wounds. Fine green birefringence patterns, characteristic of immature connective tissue, were seen in control samples viewed with polarized light. In contrast, vanadate-treated wounds showed thick yellow-orange birefringence patterns characteristics of more mature connective tissue. Using α-smooth muscle actin immunostaining, myofibroblasts were prominent in control incisions, but few were identified in vanadate-treated incisions.

Conclusions: In rat laparotomy wounds, a single application of vanadate increases wound breaking strength, through enhanced connective tissue organization. These combined data suggest topical application of vanadate immediately after fascial closure will increase wound strength, possibly reducing hernia recurrences in the repaired abdominal wall.

Introduction

In the United States, approximately 10% of patients undergoing laparotomy will develop an incisional hernia.[1,2] Incisional hernias are fraught with numerous complications such as pain, intestinal obstruction, skin ulceration, fistula, and potential compromise of the intestinal blood supply.[2] There is little consensus in the literature on which approach (laparoscopic *versus* open), implantable mesh (synthetic *versus* biologic), or suture material provides the strongest repair. Implantable materials and reoperations each have their disadvantages. Therefore, a simpler solution may be given to

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2016.08.078

make the primary repair stronger, lessening the likelihood of an incisional hernia. To do so, this would require an alteration in normal wound healing. Wound healing is a critical biological process that restores tissue integrity from loss by trauma or necrosis. The replacement of tissue is usually achieved by scarring, where a patch of connective tissue holds the edges of the defect together. A scar is not equivalent to the lost tissue in terms of function, strength, suppleness, and appearance. In most injuries, the scar is the consequence of wound repair rather than regeneration. The repair process follows a predictable sequence of cell populations that enter the repair site. Wound repair starts with the lag phase, where inflammatory cells (neutrophils and macrophages) populate the site. The proliferative phase follows, fibroblasts enter the wound site and deposit a new connective tissue matrix composed mostly of randomly organized collagen fiber bundles. During the final remodeling phase, the myofibroblast is the major cell phenotype. The myofibroblast contains stress fibers with cytoplasmic filaments that contain an isoform of α-smooth muscle (α-SM) actin.[3] During this phase, collagen fibrils condense into thicker collagen fibers and myofibroblasts eventually undergo apoptosis. This transitioning of cell types occurs in a complex milieu known as granulation tissue. Granulation tissue is the transitional connective tissue matrix that ultimately matures into the scar. Granulation tissue has a high cell density, fine collagen fibrils enriched in type III collagen, and a dense network of blood vessels necessary for the elevated metabolic needs. After cellular apoptosis, the final scar has limited vasculature, few fibroblasts, no myofibroblasts, and an unstable connective tissue matrix composed of irregular-sized collagen fiber bundles arranged in random arrays.[4] This haphazard organization of collagen fiber bundles within the scar is a major reason for its reduced tensile strength. The uniform quality of the connective tissue matrix that makes up the scar in vanadate-treated wounds is responsible for increased strength, having qualities similar to intact dermis.[5-8]

Surgical access to the abdominal cavity is typically via a midline laparotomy incision made through the linea alba. The linea alba is the aponeurosis of anterior and lateral abdominal wall musculature that extends from the xiphoid to the pubic symphysis.[9] It is composed of connective tissue structures rich in collagen. The repair of a damaged linea alba requires both the synthesis of new collagen and its subsequent reorganization into a connective tissue matrix can withstand tensile stresses from the viscera pushing against the abdominal wall. The strength of a healed laparotomy incision is weaker than the virgin linea alba.[10] In a cadaveric model, Hollinsky and Sandberg demonstrated the tensile strength of normal, uninjured epigastric regions of the linea alba ruptured at a horizontal load of 10.0 ± 3.4 N/mm^2 as compared to 6.9 ± 2.5 N/mm^2 in repaired linea alba scar tissue ($P \leq 0.001$). Scar tissue thus has a significantly reduced loading capacity than the intact ventral abdominal wall and therefore poses a permanent risk for herniation.[10]

It is well documented, in fibroblasts and other cell types, those intracellular signaling pathways can be controlled through specific tyrosine phosphorylated residues in select proteins. Protein tyrosine phosphatases (PTPs) primarily act to downregulate signaling pathways. Thus, the inhibition of PTPs could promote and or maintain the activities of native signaling pathways. In fibroblasts, PTPs modulate mitogenic signaling, regulate motility, and control mRNA levels of type I and III procollagen.[11-13] Vanadate is a broad PTP inhibitor and acts as a phosphate analogue exerting its action by binding to the transition state of specific PTPs.[14]

The purpose of this study was to demonstrate that fascial repair responds to topical vanadate therapy similar to dermis; where there is more uniform packing of collagen fiber bundles within the repair site. It is the uniform packing of collagen fiber bundles that is responsible for the doubling of dermal wound-breaking strength. This is in contrast to the increased deposition of connective tissue seen in dermal wounds treated with transforming growth factor-beta (TGF-β).[5-8,15]

Materials and methods

All animal studies followed protocols compliant with our institution's guidelines outlined in the Guide for the Care and Use of Laboratory Animals, authored by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication #86-23, Revised 1985) IACUC #2007-123.

Twelve adult female Sprague Dawley rats (350-400 g) were randomly assigned to either treatment with vanadate or normal saline (six in each group). The rats were placed into an induction chamber of 5% isoflurane anesthesia with a balance of oxygen and maintained via facemask consisting of 2-4% isoflurane throughout the surgical procedure. The abdomen of the animal was clipped and prepared with tincture of iodine. A midline abdominal skin incision was made from the xiphoid process to the pubic symphysis. The skin adjacent to the incision was undermined for approximately 1 cm in all directions off the anterior abdominal wall. Once hemostasis was obtained, the linea alba was visualized, and a full thickness incision was made into the peritoneum. The linea alba was then closed using 5-0 polydioxanone suture in a running locking fashion. A running horizontal mattress suture was started from the caudal most portions of the skin incision and continued cephalad until a skin opening of approximately 1 cm remained. At that time, a mixture of Pluronic (BASF Corp, Florham Park, NJ) with saline or Pluronic plus 300-μM sodium orthovanadate (Sigma Chemical Co, St. Louis, MO) was layered onto the repaired abdominal wall through the skin opening starting from xiphoid extending to the pubis. With the gel in a subcutaneous pocket, immediately anterior to abdominal wall, the skin incision was sutured closed. Postoperative analgesia was maintained with bupinorphine every 12 h as needed for pain, and the animals were given free access to food and water. On postoperative day 10, each rat was anesthetized with isoflurane and euthanized by an intracardiac injection of sodium pentobarbital (150 mg/kg).

The abdominal wall was harvested *en bloc*, and a 1-cm strip of tissue was cut from the superior, middle, and inferior regions in a transverse fashion. These strips of tissue were then subjected to testing using a custom built tensiometer. A Harvard apparatus PhD 2000 infusion pump (Holliston, MA) was used to provide a constant linear force (3.0×10.4 m/s). Machined aluminum posts were mounted onto the existing