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Compression therapy affects collagen type balance in hypertrophic scar

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ABSTRACT

Background: The effects of pressure on hypertrophic scar are poorly understood. Decreased extracellular matrix deposition is hypothesized to contribute to changes observed after pressure therapy. To examine this further, collagen composition was analyzed in a model of pressure therapy in hypertrophic scar.

Materials and methods: Hypertrophic scars created on red Duroc swine ($n = 8$) received pressure treatment (pressure device mounting and delivery at 30 mm Hg), sham treatment (device mounting and no delivery), or no treatment for 2 wk. Scars were assessed weekly and biopsied for histology, hydroxyproline quantification, and gene expression analysis. Transcription levels of collagen precursors COL1A2 and COL3A1 were quantified using reverse transcription-polymerase chain reaction. Masson trichrome was used for general collagen quantification, whereas immunofluorescence was used for collagen types I and III specific quantification.

Results: Total collagen quantification using hydroxyproline assay showed a 51.9% decrease after pressure initiation. Masson trichrome staining showed less collagen after 1 ($P < 0.03$) and 2 wk ($P < 0.002$) of pressure application compared with sham and untreated scars. Collagen 1A2 and 3A1 transcript decreased by 41.9- and 42.3-fold, respectively, compared with uninjured skin after pressure treatment, whereas a 2.3- and 1.3-fold increase was seen in untreated scars. This decrease was seen in immunofluorescence staining for collagen types I ($P < 0.001$) and III ($P < 0.04$) compared with pretreated levels. Pressure-treated scars also had lower levels of collagen I and III after pressure treatment ($P < 0.05$) compared with sham and untreated scars.

Conclusions: These results demonstrate the modulation of collagen after pressure therapy and further characterize its role in scar formation and therapy.

1. Introduction

Hypertrophic scars result from an abnormal wound healing response after burn or traumatic injury, surgery, or inflammation [1]. These lesions are characterized by an erythematous, raised appearance with concomitant symptoms including pruritus and pain [2,3]. Scaring can increase morbidity through debilitating contractures that impair

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activities of daily living or lead to psychosocial issues such as depression or anxiety [4–6]. Hypertrophic scars are present in up to 16% of the population and particularly affect individuals with darker skin pigmentation [7,8]. The treatment of hypertrophic scar is an area of great interest. Management strategies include silicone gels and sheeting, topical steroids, intralesional injections, radiation, laser therapy, and surgical scar correction [7,9,10]. Pressure therapy has emerged as a noninvasive and cost-effective method of hypertrophic scar treatment [11]. However, lack of standardized protocols or validated animal models has hindered the full understanding of its mechanism of action on hypertrophic scar [12,13].

At least 28 types of collagen have been defined [14–16] with collagen types I and III identified at higher proportions relative to other collagen types in normal human skin [17]. Type I collagen is the major component of the extracellular matrix (ECM), whereas type III collagen is the predominant collagen type in the healing wound [18]. Excessive collagen deposition has been implicated in the pathogenesis of hypertrophic scar. Compared with normal skin, collagen synthesis is as much as three times higher in hypertrophic scar and 20 times higher in keloids [19]. It has been shown that the regulation of type I collagen synthesis is inefficient in hypertrophic scars, resulting in a higher ratio of type I to type III collagen [20,21]. This is further worsened by the increased activity and disturbed apoptosis mechanisms of hypertrophic scar fibroblasts compared with normal fibroblasts from uninjured skin [22,23].

Pressure therapy is thought to work by decreasing blood flow to scar, resulting in collagenase-mediated collagen breakdown, hypoxia-induced fibroblast and collagen degradation, and decreased scar hydration [1,24]. Although studies on pressure treatment of scar have examined modulation of myofibroblasts [25], matrix metalloproteinases [26], and tumor necrosis factor-α [27], studies examining its direct effect on collagen are limited. The aim of this work was to determine if the application of pressure results in a measurable decrease in collagen composition and subtypes using a previously defined porcine model of hypertrophic scar.

2. Materials and methods

2.1. Animal model

All described animal work was reviewed and approved by the MedStar Health Research Institute's Institutional Animal Care and Use Committee. Juvenile castrated male Duroc swine were received and handled according to standard facility operating procedures under the animal care and use program accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and Animal Welfare Assurance through the Public Health Service.

Duroc swine were prepared and used as previously described [28]. Animals were brought to an operating suite and anesthetized using a combination of ketamine and xylazine delivered intramuscularly. Wounds were created on four red Duroc pigs using a Zimmer dermatome (Zimmer, Ltd, Swindon, United Kingdom). On each flank, a 4 × 4 inch (10.16 × 10.16 cm) wound was excised over the rib cage to a depth of 0.09 inches (0.03 in x 3 passes). A total of eight wounds (two flank wounds per pig) were created. Wounds were dressed with Mepilex Ag (Molnlycke, Gothenburg, Sweden) and changed weekly.

Wounds were observed for reepithelialization and hypertrophic scar formation. By day 70 after wound creation, all wounds were confirmed to have formed hypertrophic scars. At this point, an automatic pressure delivery system (APDS) for scar compression therapy [29] was mounted onto hypertrophic scars for treatment as previously described [28]. This device consisted of a polycarbonate enclosure that housed a wireless communication device (XBee 2.4 GHz RF modem; Digi International, Inc, Minnetonka, MN) and a compression plate for pressure delivery onto hypertrophic scar. A force sensor positioned on the compression plate allowed real-time assessment of pressure delivery and was part of a feedback mechanism to ensure pressure delivery at a preset dose. A polycarbonate base was secured to surrounding skin using MYO/WIRE II Sternotomy Suture (A&E Medical Corporation, Durham, NC) followed by attachment of the APDS to this base. Protective padding, casting material, and a custom-fitted 5-mm-thick neoprene vest [30] were then placed to ensure further protection of the animal and device.

2.2. Experimental design

Developed scars received pressure treatment (pressure device mounting and pressure delivery, $n = 4$), sham treatment (pressure device mounting and no pressure delivery, $n = 2$), or no treatment at all (no pressure device mounting or pressure delivery, $n = 2$). Pressure therapy was set at 30 mm Hg of constant pressure for 2 wk.

Scar assessments were performed just before pressure initiation at week 0 (day 70 after wound creation), after 1 and 2 wk of pressure therapy, and 1 wk after treatment removal at week 3. The APDS remained mounted for the entirety of the 2 wk except when it was removed briefly for the week 1 scar assessment. Assessments included evaluation of scar maturation, biopsy procurement, and digital photography. Punch biopsies (3 mm) were taken from pretreated scars and at weekly assessments. Biopsies were then placed in formalin for histology or AllProtect Tissue Reagent (Qiagen, Valencia, CA) for RNA isolation.

2.3. Histology

Punch biopsies were fixed in 10% formalin, embedded in paraffin, and sectioned to a thickness of 6 μm on a microtome (Leica, Wetzlar, Germany). Slides were deparaffinized using xylene and rehydrated through an ethanol gradient. Staining was then performed by Masson trichrome or immunofluorescence using previously described protocols [28,31]. For immunofluorescence, primary antibodies included monoclonal anti-mouse collagen type I (Abcam, Cambridge, United Kingdom) and monoclonal anti-mouse collagen type III (Abcam) at a 1:500 dilution. A polyclonal goat anti-mouse IgG-Cy3 (Abcam)–conjugated secondary antibody at a dilution of 1:100 was also used.

ImageJ (version 1.48, NIH, Bethesda, Maryland) imaging software was used to analyze Masson trichrome digital images and quantify the amount of collagen per high-powered