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Collagen Remodeling and Suburethral Inflammation Might Account for Preserved Anti-incontinence Effects of Cut Polypropylene Sling in Rat Model

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Abstract

OBJECTIVES—To determine whether the inflammatory responses or collagen remodeling in suburethral tissue could have contributed to the preserved anti-incontinence effects of a cut polypropylene sling.

METHODS—Stress urinary incontinence was created in 60 age-matched female Sprague-Dawley rats that were subsequently randomized into 3 equal-size groups according to surgical procedure: placement of a vaginal suburethral sling, placement of a vaginal suburethral sling in which the suburethral portion of the sling was immediately cut, and sham surgery without placement of the sling. In a previous study, the leak point pressure measurements were obtained on these rats 6 weeks after surgery. The rats were then killed, the urethrovaginal tissue was harvested (cross-section of the entire urethra and anterior vagina) from 30 animals (10 from each experimental group), and the tissue was stained with hematoxylin-eosin and Masson's trichrome for histopathologic studies and picrosirius red for collagen fibers.

RESULTS—As previously published, the median leak point pressures were similarly and significantly increased in the rats in the intact and cut sling groups compared with those in the sham surgery group. The inflammatory mediators and interstitial edema were similarly increased in the intact and cut sling specimens compared with the sham surgery specimens. Under polarized light, picrosirius red-stained specimens from the sham surgery animals appeared to be composed of collagen that predominately birefringed red to yellow (typical of type I collagen). The picrosirius red-stained cut and intact sling specimens appeared to contain collagen fibers that predominately birefringed green (typical of type III collagen).

CONCLUSIONS—Histologic changes, including inflammation, localized edema, and differential collagen remodeling, might contribute to the preserved anti-incontinence mechanisms of cut or intact polypropylene slings observed clinically.

It has been estimated that >126 000 surgeries are performed each year for stress urinary incontinence (SUI) in women.¹ Sling procedures have become the mainstay of surgical treatment for SUI.² Although the sling procedure results in high success rates, it can also lead to complications, including voiding dysfunction and urinary retention.^{3,4} Several series have demonstrated preservation of continence in 80% of patients, even after cutting the sling suburethrally to reverse iatrogenic obstruction.^{5–7} Although histologic analyses have demonstrated evidence of inflammation and fibrosis after implantation of different sling types,^{8–10} it is unclear whether these histologic changes contribute to the preservation of continence after cutting or removing slings. Furthermore, it is also unknown whether specific evidence exists of collagen remodeling.

Although the number of collagen subtypes is approximately 21, the endopelvic fascia and ligaments contiguous with the urethra, bladder, and vagina are comprised predominately of collagen types I and III.^{11–14} Collagen type I is the major fibril-forming collagen.¹⁵ It polymerizes into the largest fibers and is the primary determinant of tensile strength. Collagen type III predominates in tissues such as blood vessels and is thought to impart flexibility and distensibility to tissues.

In a rat model of SUI, we have previously shown that placement of a suburethral polypropylene sling in a tension-free manner resulted in restoration of leak point pressures (LPPs) to those of control animals without SUI.^{16,17} Using that model, we have previously reported the results of a randomized trial in which rats with SUI were assigned to either placement of an intact suburethral sling (intact sling) or a suburethral sling that was immediately cut after placement at the suburethral portion (cut sling), or vaginal dissection and needle passage without sling placement (sham surgery).¹⁸ Examination of the LPPs in these rats at 6 weeks after surgery revealed that the rats with either a cut or an intact sling had equally and significantly improved LPPs compared with the rats that had undergone sham surgery. The results of that study indicated in contrast to the conventional view, preservation of the anti-incontinence effects of slings might not be solely dependent on the intactness of the suburethral portion of the sling. The aim of the present study was to determine whether the inflammatory responses or collagen remodeling in the suburethral tissue could have contributed to the preserved anti-incontinence effects of a cut polypropylene sling.

MATERIAL AND METHODS

The Institutional Animal Care and Use Committee approved this study. SUI was created in virgin female Sprague-Dawley rats by a standardized bilateral pudendal nerve transection, as previously reported.¹⁸ The rats were then randomized into 3 groups of equal size according to the surgical procedure: placement of a vaginal suburethral sling, placement of a vaginal suburethral sling in which the suburethral portion of the sling was immediately cut, and sham surgery without placement of the sling. At 6 weeks after surgery, the rats underwent

anesthetized LPP testing by examiners unaware of the treatment group using objective techniques established by Cannon and Damaser.¹⁹ After completion of LPP testing, the rats were killed, the urethrovaginal tissue was harvested (cross-section of the entire urethra and anterior vagina), the tissues were immersion-fixed, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin-eosin, Masson's trichrome, and picrosirius red. Qualitative histologic analyses of 10 hematoxylin-eosin and Masson's trichrome stained specimens from each treatment group (30 specimens total) were done under light microscopy by examiners unaware of the treatment groups.

Digital images of the 10 picrosirius red-stained specimens from each treatment group (30 specimens total) were obtained using an upright, linearly polarized light microscope (Leica DMR, Leica-Microsystems, Heidelberg, Germany). The quantification of the relative collagen content was assessed by analyzing the captured images (1.656 $\times$ 1.312-mm field size) using Image-Pro Plus, version 5.0, software (Media Cybernetics, Silver Springs, MD). A color-segmentation profile was generated using the green-orange/red collagen birefringence of a picrosirius red-stained lung sample as the standard. The color-segmentation profile was then applied to a manually selected region of interest in each sample. The region of interest (suburethra) was the fibromuscular connective tissue between the urethra and vaginal epithelium, which was further standardized with the striated muscle of the urethra as the superior and lateral borders and vaginal epithelium as the inferior borders. Within each region of interest, the area containing birefringent collagen was measured and recorded as the area of collagen type I or III in square microns. Because the region of interest (suburethra) was not a uniform size in all the specimens, the percentage of birefringent collagen per unit area was calculated.

The potential differences between treatment groups were analyzed using the Kruskal-Wallis test, with $P > .05$ indicating a significant difference. The Tukey multiple comparison post hoc test was used to evaluate the significant differences between the individual treatment groups. The quantitative data are presented as the median, interquartile range, and maximal and minimal values. Statistical analyses were performed using JMP, version 5.0.1 (SAS Institute, Cary, NC).

RESULTS

Three rats in the cut sling group died of unknown reasons, with necropsy revealing unremarkable findings. Therefore, LPP measurements were obtained on the remaining 17 rats in the cut sling group, 20 in the sham surgery group, and 20 in the intact sling group. As previously demonstrated, the median LPP values were similarly and significantly increased in rats in the intact and cut sling groups compared with the rats in the sham surgery group (27.9 cm H₂O, interquartile range [IQR] 26.1–32.7; 24.9 cm H₂O, IQR 23.3–34.1; 20.7 cm H₂O, IQR 17.6–24.6; respectively, $P > .001$).¹⁶

Because of the initial difficulties in tissue harvest and preservation, only 10 intact and complete specimens were available from each experimental group for histologic analysis. Although individual histologic variations existed within the same treatment groups, the inflammatory mediators and interstitial edema were similarly increased in the cut and intact

sling animals compared with the sham surgery rats (Fig. 1). The specimens from the rats with cut and intact slings contained vacuoles that resulted from the fragmented polypropylene sling fibers. These vacuoles were surrounded by fibrosis and lymphocytes (Fig. 1D). Specimens from the rats with cut and intact slings retained less of the aniline blue dye in the Masson's trichrome stain, indicating edema (Fig. 2). As a result, the suburethral areas in these rats were enlarged compared with the sham surgery animals.

Under linearly polarized light, picrosirius red-stained specimens from the sham surgery animals appeared to be composed of collagen that predominately birefringed red to yellow (typical of type I collagen). In contrast, the picrosirius red-stained cut and intact sling specimens appeared to contain collagen fibers that predominately birefringed green (typical of type III collagen; Fig. 3). Analysis of the suburethral region from the digital images of these specimens confirmed a significantly decreased percentage of area that birefringed red to yellow, typical of type I collagen, from the cut and intact sling groups compared with the sham surgery group (0.19%, IQR 0.13%–0.25%; 0.27%, IQR 0.24%–0.34%; 0.52%, IQR 0.34%–0.60%; respectively, $P > .0001$). In contrast, specimens from the cut and intact sling groups had a significantly greater percentage of area that birefringed green, typical of type III collagen, than the sham surgery group (0.33%, IQR 0.31%–0.35%; 0.32%, IQR 0.26%–0.35%; 0.14%, IQR 0.11%–0.33%; respectively, $P > .0008$). Furthermore, although no statistically significant correlations were found between the LPPs and collagen content, increased LPPs were associated with a decreased percentage of area of type I-like collagen fibers and an increased percentage of area of type III-like collagen fibers.

COMMENT

Traditional slings and mid-urethral sling-like procedures such as the tension-free vaginal tape are the most common anti-incontinence procedures performed for SUI.² The anti-incontinence mechanism behind slings is thought to be secondary to compression of the urethra during conditions of increased intra-abdominal pressure. However, it is unclear which factors are responsible for this mechanism. In published series, most patients who required cutting or removal of slings for postoperative voiding dysfunction were still continent afterward.^{5–7} Driven by those clinical findings, we have previously demonstrated that the LPPs were similarly and significantly increased in animals with intact and cut slings compared with sham surgery animals.¹⁶ No statistically significant differences were found in the LPPs between the rats with cut or intact slings.

In the present study, we found histologic evidence of increased localized edema and inflammation in the rats that also had an increase in LPPs. Also, significant differences were found in the quality of the collagen remodeling in the cut and intact sling groups compared with the sham surgery group. Specifically, the specimens from the cut and intact sling groups demonstrated a similarly increased percentage of area of collagen fibers that had birefringence typical of type III collagen and a decreased percentage of area of collagen fibers that had birefringence typical of type I collagen compared with the specimens from the sham group. Because the sham surgery rats had undergone the identical surgery and dissection without placement of the sling, the histologic differences found in the cut and intact sling specimens were likely induced by the polypropylene sling material itself,

regardless of sling's intactness and were not solely a result of postoperative healing. It therefore appears that increased LPPs are associated with the histologic changes of inflammation and collagen remodeling resulting from the polypropylene sling, whether it is intact or not.

We used the picrosirius red-polarized light method to differentiate the collagen subtypes and the relative amounts according to the organization of the fibers and arrangements of the collagen molecules. Collagen type I forms closely packed thick fibers (2–10 μm in diameter) and exhibits birefringence as an intense yellow to red color. Collagen type III forms loose thin fibers (0.5–1.5 μm in diameter) and has a weak green birefringence.²⁰ Junqueira et al.^{21,22} first described analyzing picrosirius red-stained specimens using polarized light to evaluate collagen in different types of tissue. Picrosirius red is an acidic dye that reacts with the basic amino acids that are found within collagen molecules. This reaction leads to the alignment of picrosirius red molecules parallel with the long axes of the collagen molecules, resulting in an enhancement of the normal birefringence specific to different types of organized aggregates of collagen fibers.²³

Although the picrosirius red dye reacts with other structures that contain basic proteins, these proteins do not trigger the birefringence, because they lack the organized uniform molecular structure of collagen. Therefore, the different intensities and colors of birefringence exhibited by the different types of collagen enhanced by picrosirius red staining are thought to be dependent on the physical organization of the different types of collagen fibers. Relative amounts of collagen subtypes can be determined by calculating the amount of collagen at each pixel of a digitized micrograph.²⁴ The findings of differential tissue localization of collagen subtypes observed with this technique have also been confirmed using immunohistochemical and biochemical methods.^{25–27} However, as previously demonstrated in wound healing studies, it is possible to overcategorize all newly deposited fibers as collagen type III because many of these newly deposited fibers are initially loose thin fibers in the process of polymerizing into larger fibers.²³ Therefore, in this study, we could most accurately conclude that the collagen remodeling 6 weeks after the introduction of a cut or an intact sling consisted primarily of a shift in the type of collagen from type I-like fibers to type III-like fibers.

During the maturation and remodeling phase of wound healing, an early and increased initial deposition of collagen type III occurs.¹⁵ Because the rats in this study were tested and subsequently killed for histologic examination at one point (6 weeks after surgery), it is possible that the observed differences in collagen remodeling between the experimental groups could be a transient effect secondary to the differential healing process and wound maturation caused by the polypropylene sling. Immature collagen type III might be replaced by type I collagen later. However, the differences in LPPs were also obtained 6 weeks postoperatively and were associated with the histologic findings, suggesting an association between collagen remodeling due to the sling (either cut or intact) and restoration of LPP.

CONCLUSIONS

The specimen from rats with cut or intact slings demonstrated histologic changes such as chronic inflammation, localized edema, and differential collagen remodeling, which might contribute to the restoration of LPPs observed in these animals compared with the sham surgery rats. These histologic changes might contribute to the preserved anti-incontinence mechanisms of cut or intact polypropylene slings observed clinically.

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Figure 1.

Hematoxylin-eosin stained urethrovaginal specimens from rats with (A) cut sling, (B) intact sling, and (C) sham surgery. (D) Vacuoles that resulted from fragmented polypropylene sling fibers in cut sling specimen surrounded by fibrosis (arrowhead) and lymphocytes (arrow). Similar vacuoles seen in specimens from rats with intact slings. V, vacuoles from fragmented sling fibers; U, urethra. Fig. A–C, bar > 500 μ m; Fig. D, bar > 80 μ m.

Figure 2.

Masson's trichrome stained urethrovaginal specimens from rats with (A) cut sling, (B) intact sling, and (C) sham surgery. Smooth muscle and collagen stained light pink and blue, respectively. V, vacuoles from fragmented sling fibers; U, urethra. Bar > 500 μm.

Figure 3.

Picrosirius red-stained specimens from different treatment groups viewed with polarized light. Images from rats with (A) cut sling, (B) intact sling, and (C) sham surgery. Suburethral area, bordered superiorly and laterally by striated urethral muscles and inferiorly by vaginal epithelium, was analyzed (outlined in white). Collagen type I birefringes yellow to red. Collagen type III birefringes green. V, vacuoles from fragmented sling fibers; U, urethra. Bar > 500 μ m.