

PERIURETHRAL INFLAMMATORY AND COLLAGEN REMODELLING MAY ACCOUNT FOR PRESERVED ANTI-INCONTINENCE EFFECTS OF A CUT SLING IN A RAT MODEL

Hypothesis / aims of study

It is estimated that more than 126,000 surgeries are performed each year for stress urinary incontinence (SUI) with sling procedures as the mainstay of surgical treatment. While sling procedure results in high success rates, it can also lead to complications including voiding dysfunction and urinary retention. Several series have demonstrated preservation of continence in approximately 80% of patients even after cutting the sling suburethrally to reverse iatrogenic obstruction. [1] It is thought that preservation of continence even in cut or removed slings result from fibrosis induced by the sling material. Although previous histologic analyses have demonstrated evidence of inflammation and fibrosis after implantation of different types of slings, it is unclear if there is specific evidence of collagen remodelling. Junqueira et al. first described analyzing Picrosirius red stained specimen using polarized light to evaluate collagen in different types of tissue. [2] Type I collagen form thick fibrils and birefringes as an intense orange to red colour. Type III collagen form thin fibers and has weak green birefringence. Although we have previously confirmed that cutting the sling suburethrally does not change its anti-incontinence effects in a validated rat model of SUI [3], we have not addressed potential etiologies of the continued anti-incontinence effects. Our aim was to determine if inflammatory responses or collagen remodelling in the urethro-vaginal tissue could have contributed to the preserved anti-incontinence effects of cut polypropylene sling.

Study design, materials and methods

Institutional Animal Care and Use Committee approval was obtained. SUI was created in 60 age-matched female Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250 to 300 gm by previously established method of pudendal nerve transection. The rats were then randomized into three equally sized 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. Vaginal suburethral sling procedure was performed based on previously established methods and involved placement of a 3 x 20 mm polypropylene mesh. The sham surgery group underwent the vaginal dissection and needle passage without placement of the actual sling. Six weeks after surgery, the rats underwent placement of a suprapubic catheter for urodynamic measurements including leak point pressures (LPP) using previously published methods. After completion of LPP testing, the rats were euthanized, urethro-vaginal tissue harvested (cross-section of entire urethra and anterior vagina), immersion-fixed, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin-eosin, Masson's trichrome, and Picrosirius red stains. Qualitative histologic analyses of thirty hematoxylin-eosin and Masson's trichrome stained specimens (ten from each treatment group) were made using a light microscope. Digital images of thirty Picrosirius red stained specimens were obtained using an upright polarized light microscope (Leica DMR, Leica-Microsystems, Heidelberg, Germany). Quantification of the relative collagen content was assessed by analyzing the captured images (1.656 mm x 1.312 mm field size) using Image-Pro Plus 5.0 software (Media Cybernetics, Silver Springs, MD). A colour-segmentation profile was generated using the green-orange/red collagen birefringence of a Picrosirius red stained lung sample as the standard. The colour-segmentation profile was then applied to a manually selected region of interest in each sample. The region of interest (urethro-vaginal tissue) was the fibromuscular connective tissue between the urethra and vaginal epithelium, which was further standardized with the striated muscles of the urethra as the superior and lateral borders and vaginal epithelium as the inferior borders. For each sample, the areas containing the birefringent collagen was measured and recorded as amount of collagen in square microns. As the region of interest was not a uniform size in all the specimens, the percentage of birefringent collagen/ area was calculated. Potential differences between treatment groups were analyzed using the Kruskal-Wallis test. If the Kruskal-Wallis test was significant at the 0.05 level, Tukey multiple comparisons test was performed to evaluate which treatment groups differed. Statistical analyses were performed using JMP 5.0.1 (SAS Institute, Cary, NC).

Results

Median LPP values were similarly and significantly increased in animals with cut and intact vaginal slings compared with animals with sham surgeries (24.9 cmH₂O, 27.9 cmH₂O, 20.7 cmH₂O, respectively, P < 0.001). Although individual histologic variations existed within the same treatment groups, inflammatory mediators and interstitial edema were similarly increased in cut and intact sling animals compared with sham surgery animals. Specimens from animals with cut and intact slings contained vacuoles that resulted from the fragmented polypropylene sling fibers. These vacuoles were surrounded by fibrosis and lymphocytes. Specimen from animals with cut and intact slings retained less of the aniline blue dye in the Masson's trichrome stain, indicating edema. Under polarized light, specimens from the sham surgery animals appeared to be composed of collagen that predominately birefringed red to orange (typical of type I collagen) whereas cut and intact sling specimens appeared to contain collagen fibers that predominately birefringed green (typical of type III collagen). Specifically, the median percentage of collagen type I over the analyzed area in the cut and intact sling groups were similarly and significantly decreased compared with the sham surgery group (19.0%, 26.9%, 52.1%, respectively, P < 0.0001), while the median percentage of collagen type III over the analyzed area in the cut and intact sling groups were similarly and significantly increased compared with the sham surgery group (33.0%, 31.8%, 14.0%, respectively, P = 0.0008).

Interpretation of results

In this rat model of SUI, animals with cut and intact slings had restoration of urinary continence as measured by similar and significantly increased leak point pressures compared with animals that underwent sham surgery. There was evidence of localized edema and chronic inflammatory reaction in animals that received suburethral slings (cut or intact) 6 weeks after the initial surgery. There was also evidence of collagen remodelling with similar and significantly decreased collagen type I and increased collagen type III in cut and intact sling animals compared with sham surgery animals.

Concluding message

Incision of the sling at its suburethral section did not affect the anti-incontinence impact of the vaginal sling, as measured by LPP, in a rat model of SUI. The preserved anti-incontinence effects may be due to the presence of periurethral inflammation along with collagen remodelling as evidenced by an increase in collagen type III and decrease in collagen type I.

References

1. Urology (2003) 62; 714-718.
2. Histochem I (1979) 11; 447-455.
3. J Urol (2005) 174; 771-775.

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Institutional Animal Care and Use Committee