Reduced Flow after Tubularized Incised Plate Urethroplasty—Increased Fibrogenesis, Elastin Fiber Loss or Neither?

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Abbreviations and Acronyms

CC = corpus cavernosum

ECM = extracellular matrix

PCR = polymerase chain reaction

TIP = tubularized incised plate urethroplasty

TIPG = tubularized incised plate urethroplasty with inlay preputial graft

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Purpose: Low urinary flow rates are common after tubularized incised plate urethroplasty but the etiology remains unclear and may be related to low urethral compliance due to abnormal collagen concentrations and/or fewer elastic fibers in the healed urethral plate. We hypothesized that inserting a preputial mucosal graft over the dorsal raw area after the midline incision may avoid scarring and improve urethral compliance.

Materials and Methods: Adult rabbits were submitted to tubularized incised plate urethroplasty with or without inlay preputial graft according to a previously described protocol. Tissular concentrations of collagens I, III, IV, VI, VIII and XIII were measured. Histomorphometric analysis was used to quantify elastic fibers in the urethra. Tubularized incised plate urethroplasty with and without inlay preputial graft was compared to normal rabbit urethras (controls).

Results: mRNA concentrations for collagens I, II and XIII were similar between controls and operated rabbits. The proportions between collagens I and III were 1.05, 0.87 and 1.21, respectively, in controls and animals undergoing tubularized incised plate urethroplasty with and without inlay preputial graft. mRNA concentrations for collagen IV and collagens VI/VIII tended to be higher and lower, respectively, in the operated urethras, despite showing statistical significance only for collagen VIII in animals undergoing tubularized incised plate urethroplasty with inlay preputial graft vs controls ($p = 0.02$). The operated animals did not demonstrate a reduced number of elastic fibers in the urethral tissues compared to controls.

Conclusions: Elastic fiber number and distribution were similar between tubularized incised plate urethroplasty cases and controls, suggesting that decreased concentrations of elastic fibers do not explain the reduced urethral compliance after tubularized incised plate urethroplasty. The raw area determined by the dorsal urethral incision regenerated after standard tubularized incised plate urethroplasty, while cicatrization with fibrosis occurred in correspondence to the grafted areas after tubularized incised plate urethroplasty with inlay preputial graft.

Key Words: collagen, elastin, hypospadias, skin transplantation, urethra

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TUBULARIZED incised plate urethroplasty is widely used for hypospadias repair.[1] Abnormal urinary flow rates are common postoperatively, despite the absence of anatomical urethral obstruction in most cases.[2] Previous experimental results indicate that tubularized incised plate urethroplasty does not induce extensive urethral fibrosis,[3–5] but modifications of the collagen distribution or loss of elastic fibers in the operated area are still possible.

We studied these aspects further in a previously described animal model,[2] departing from the hypothesis of a loss of urethral compliance in TIP models, focusing instead on the possibility of fibrosis, differential collagen distribution or paucity of elastic fibers after cicatrization of the median incision. We also studied how the addition of a free graft over the raw dorsal area after the midline incision modified urethral cicatrization. Our objectives were to evaluate tissular concentration of different collagens, and to quantify and describe the distribution of elastic fibers in normal urethras and TIP and TIPG models.

METHODS

The experimental protocol was approved by our institutional animal care committee (REB 1000007883). All handling and procedures were performed following the Canadian Council on Animal Care guidelines.

A total of 25 adult male 3 to 3.5 kg New Zealand rabbits were kept in individual cages receiving a standard rabbit diet, water ad libitum and routine care, and preanesthetized with ketamine and acepromazine. Anesthesia was accomplished by a mixture of halothane, nitrous oxide and oxygen through a mask and penile block with 2% lidocaine.

Animals were divided into 3 groups. Group 1 consisted of 9 nonoperated normal male adult rabbits (controls). Group 2 included 8 animals that underwent segmental TIP after resection of the ventrolateral portion of the urethral wall (not adherent to the CC), leaving approximately half of the urethral circumference, followed by a midline dorsal incision (limited to the adventitia of the CC) and ventral tubularization (single layered continuous polydioxanone 6-zero suture over a 10Fr catheter that was removed immediately after finishing the procedure). The operated segment was 2.5 cm long, beginning 1 cm proximal to the glans (fig. 1). Group 3 consisted of 8 animals undergoing the same procedure as group 2, except a mucosal preputial graft was placed over the incised area, sutured to the edges of the incision and quilted to the corporeal albuginea with interrupted 7-zero polyglactin sutures.

The animals received routine care after recovering from anesthesia. Analgesics were not required postoperatively. All animals were observed for 6 weeks and then sacrificed using anesthetic overdose.

Penile dissection and degloving was done immediately after death. Urethral compliance was evaluated by measuring tension in an isolated urethral segment after injections of specific volumes of air, as reported previously.[6] A 3 cm urethra plus CC segment distal to the penopubic junction was isolated and sectioned in 3 equal parts, each containing a segment of operated urethra in groups 2 and 3. One segment was selected for histological studies, preserved in phosphate buffered formalin and sectioned (4 μm thick sections). Four sections for each animal were stained using hematoxylin and eosin (routine histological evaluation), Movat pentachrome (specific for elastic fibers), Masson trichrome and picrosirius red. The last two staining methods are specific to evaluating collagen deposition. A stained section for each rabbit was photographed using MIRAX Viewer software (Carl Zeiss, Thornwood, New York). Using 20× magnification, 4 sections were selected for elastin fiber counting and tissue description, starting from the mucosa inward, with section 1 being median dorsal, 2 and 4 lateral, and 3 median ventral (fig. 2). The selected areas were uploaded for manual counting of elastic fibers using a grid mask tool, spaced 40 × 40 (Image-Pro® Plus software, version 4.5.0.29).

Another urethral segment was used for PCR. The tissue was washed with saline, immediately frozen in dry ice and kept in a freezer at −8C. Measurement of collagen mRNA was done for collagens I, III, IV, VI, VIII and XIII. RNA corresponding to each collagen was obtained using oligo-dT primers and reverse transcriptase (Invitrogen™). The primers were custom designed and tested before use. DNA was induced with TRIzol® solution. Normalization of the results used the reference genes b actin and HPRT.

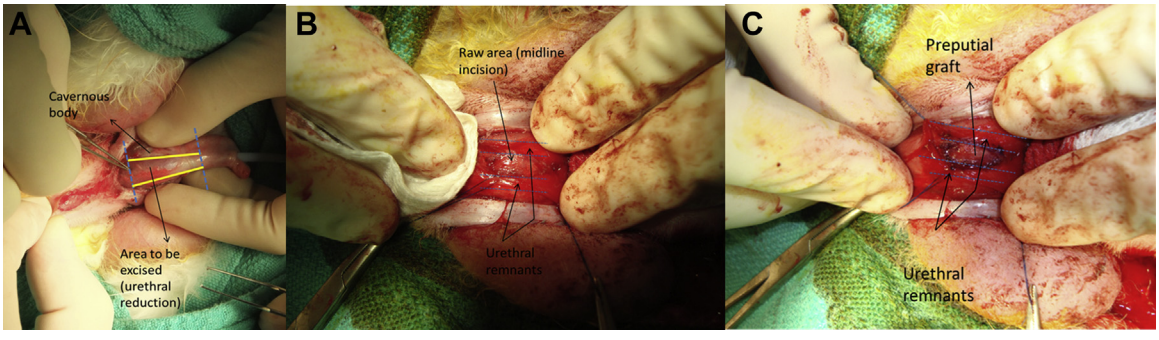

Figure 1. *A,* markings for urethral reduction (degloved penis). *B,* TIP model before tubularization. *C,* TIPG model before tubularization.