Dextranomer/Hyaluronic Acid Copolymer Implant for Vesicoureteral Reflux: Role of Myofibroblast Differentiation

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Purpose: Dextranomer/hyaluronic acid implantation is associated with a granulomatous inflammatory reaction, replaced by fibrosis. Appearance of myofibroblasts is considered a crucial event in fibrosis, and CD68 positive cells and other factors are implied in their activation. Mast cells are a source of these factors and tryptase can induce fibroblast to express α-smooth muscle actin, which is characteristic of myofibroblasts. We evaluated histological changes in refluxing ureters treated with dextranomer/hyaluronic acid and immunolocalized CD68 positive cells, tryptase mast cells and myofibroblasts.

Materials and Methods: We performed histological, histochemical and immunohistochemical analyses in 22 refluxing ureters treated with dextranomer/hyaluronic acid in comparison with 17 refluxing ureters who underwent ureteral reimplantation but did not receive endoscopic bulking agent. We used CD68 antibody for monocytes/macrophages and epithelioid cells, mast cell tryptase mouse antibody for mast cells, and α-smooth muscle actin and vimentin antibodies for myofibroblasts. The area of the ureteral lumen in dextranomer/hyaluronic acid treated and untreated ureteral endings was measured.

Results: Sirius red documented a major grade of histological lesions in dextranomer/hyaluronic acid treated refluxing ureters. CD68 and tryptase mast cell staining showed a significant enhancement of positive cells in dextranomer/hyaluronic acid treated refluxing ureters. Immunostaining for α-smooth muscle actin and vimentin displayed a myofibroblastic invasion in dextranomer/hyaluronic acid. Measurement of surface in treated refluxing ureters was significantly less than in untreated refluxing ureters.

Conclusions: Our data documented a recruitment of CD68 and tryptase positive cells, abnormal accumulation of collagenous stroma and successive extracellular matrix remodeling through differentiation of myofibroblasts. Myofibroblasts might provoke tissue contraction, decreasing the ureteral diameter and modifying the ureteral length-to-diameter ratio, preventing urine reflux.

Key Words: CD68 antigen, human; dextranomer-hyaluronic acid copolymer; endoscopy; mesangial cells; vesico-ureteral reflux

Abbreviations and Acronyms

α-SMA = α-smooth muscle actin
Dx/HA = dextranomer/hyaluronic acid copolymer
ECM = extracellular matrix
HPF = high power field
RU = refluxing ureteral ending
SD = standard deviation
VUR = vesicoureteral reflux

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ENDOSCOPIC injection of a bulking agent was pioneered in 1981 as an alternative to conservative medical treatment and surgical ureteral reimplantation in patients with vesicoureteral reflux.[1] Different substances have been investigated, and polytetrafluoroethylene, silicone and cross-linked bovine collagen have been well studied. Concerns re-

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garding the safety and efficacy of these agents have precluded their widespread use.

Dx/HA, approved by the Food and Drug Administration in 2001 for the treatment of VUR, was the first endoscopic agent available for this indication in the United States. This endoscopic bulking agent consists of dextranomer (cross-linked dextran) microspheres 80 to 250 μm in diameter in a nonanimal, stabilized hyaluronic acid gel. The physicochemical properties of Dx/HA make it ideal for treating VUR because it is biocompatible, biodegradable and nonmigratory, exhibits no signs of mutagenesis and has a good safety profile.[2,3] Dextranomer, which is the main bulking agent, is only slowly degraded by hydrolysis, whereas the hyaluronic acid matrix acts primarily as a transport medium and disappears from the body within several weeks. After submucosal injection the dextranomer microspheres stimulate collagen synthesis and fibroblast ingrowth in the degrading hyaluronic acid matrix, consolidating the implant within the bladder wall through endogenous tissue augmentation.

It is reported that Dx/HA treatment is associated with a granulomatous inflammatory reaction, as demonstrated by multinucleated giant cells that are subsequently replaced by fibrosis.[2] From the biological point of view some fibroblasts express features of smooth muscle differentiation. These smooth muscle-like fibroblasts, referred to as myofibroblasts,[4] can be identified by certain characteristic features of the cytoskeleton, particularly the expression of α-SMA.[5] It is known that myofibroblasts have a major role in inflammatory responses through their production of growth factors, cytokines and soluble mediators. Appearance of myofibroblasts is considered a critical event in the evolution of fibrosis, and activated macrophages and other specific factors are implied in their activation.[6,7] Mast cells are a potential source of some of those factors, and in particular mast cell tryptase can induce normal human dermal fibroblasts to express α-SMA, a distinguishing characteristic of differentiated myofibroblasts.[8] Moreover, interactions of mast cells with their ECM are crucial events for tissue specific migration, localization and function, since mast cells circulate as immature precursor cells and home to tissue adhering to hyaluronic acid coated surface.[9]

We evaluated the histological changes in refluxing ureters treated with Dx/HA, with respect to immunolocalized CD68 positive cells, tryptase mast cells and myofibroblasts. We also estimated their importance in the biological mechanism of action of Dx/HA.

MATERIALS AND METHODS

We performed retrospective histological, histochemical and immunohistochemical analyses of 15 patients (9 boys and 6 girls, age 18.1 $\pm$ 5.5 months) with grade III or greater VUR who received 1 implant of 0.7 to 0.9 ml Dx/HA, for a total of 22 RUs. The patients had persistent reflux 94 to 115 days (102 $\pm$ 8) after initial unsuccessful endoscopic treatment, defined as persistent grade III or greater VUR on voiding cystourethrography, and underwent ureteral reimplantation using the Cohen technique. VUR was recorded as grade III in 4 RUs, IV in 12 and V in 8. For comparison 11 controls (7 boys and 4 girls, mean $\pm$ SD age 20.4 $\pm$ 4.9 months) were enrolled in the study, for a total of 17 RUs affected by grade III or greater VUR. Controls underwent ureteral reimplantation using the Cohen technique but did not receive any type of endoscopic bulking agent. In controls VUR was recorded as grade III in 3 RUs, IV in 9 and V in 5. Indications for open surgery were poor parental compliance with treatment and reflux nephropathy. The control group was matched with the Dx/HA treated group for age, gender and reflux grade. The duration of antibiotic prophylaxis was similar in the treated and control groups.

We used colored sutures inserted into the distal ureteral endings to identify the distal part of the ureters that were used for the histological, immunohistochemical and morphological evaluations. The resected ureteral segments (about 3 mm) were fixed in 10% neutral formalin and embedded in paraffin. Paraffin blocks were labeled with a numerical code to ensure blinding to clinical data, and 4 μm thick sections were processed for routine histological analysis, and stained with hematoxylin and eosin.

For immunohistochemical evaluation parallel paraffin sections were heated in citrate buffer (antigen retrieval buffer, pH 6.0, Dako, Glostrup, Denmark) for 30 minutes in a high-powered microwave oven (400 W) to retrieve the antigens. After rinsing in phosphate buffered saline (pH 7.4) endogenous peroxidase activity was blocked in 3% H2O2 for 5 minutes. For immunohistochemical staining we used CD68 antibody (clone KP1, Dako) diluted 1:150 for identification of monocytes/macrophages and epithelioid cells, mast cell tryptase mouse antibody (clone AA1, Abcam Inc, Cambridge, Massachusetts) diluted 1:50 for identification of mast cells, and α-SMA (clone 1A4, Dako) and vimentin antibodies (clone V9, Dako) diluted 1:100 for identification of myofibroblasts. CD68, α-smooth muscle actin and vimentin antibodies were incubated overnight at 4C, while mast cell tryptase antibody was incubated at room temperature for 30 minutes.

After being rinsed in phosphate buffered saline the slides were reincubated for 10 minutes at room temperature with a biotinylated link universal secondary antibody (LSAB®+ System-HRP, Dako, Carpinteria, California) and developed in 3.3′-diaminobenzidine-HCl (VIP, SK 4600m, Vector® Laboratories, Burlingame, California). A weak hematoxylin nuclear counterstaining was then applied, and the sections were dehydrated and mounted. Parallel negative controls were processed by omitting the primary antibody incubation. For histochemical analysis after dewaxing and hydration parallel sections of the specimens were stained with sirius red for collagen stains.