Histopathological Changes Associated With Dextranomer/ Hyaluronic Acid Injection for Pediatric Vesicoureteral Reflux

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Purpose: Few studies have examined the medium and long-term histological changes associated with periureteral injection of dextranomer/hyaluronic acid copolymer (Deflux®). We present the results of a histological review of a series of distal ureteral excisions in patients undergoing ureteroneocystostomy after failed dextranomer/hyaluronic acid injection.

Materials and Methods: All patients undergoing ureteroneocystostomy after failed dextranomer/hyaluronic acid injection(s) at 1 institution were eligible for this study. Excised ureteral segments were histologically examined by a single urological pathologist. An immunohistochemical battery was used for each specimen, including hematoxylin and eosin, CD3, CD20, MIB-1 and trichrome stains. Pathological criteria included the presence, location and intensity of fibrosis, giant cell reaction, chronic inflammation, free dextranomer/hyaluronic acid, and CD3, CD20 and MIB-1 staining. Pathological features were correlated with the time from injection to surgical excision.

Results: The ureters of 16 children with a mean age of 4.5 years were examined. Median time from injection to implant excision was 8 months. Giant cell reaction was present in 94% of patients and it was typically located in the serosa. No histological or immunophenotypical feature correlated with the duration of implantation except CD3+ and CD20+ lymphocyte counts, which increased with time from injection ($p = 0.06$ and 0.02, respectively).

Conclusions: Dextranomer/hyaluronic acid appears to be stable and safe for use after 3 to 22 months of followup of subureteral injection. The periureteral inflammatory reaction increases with time, although no increases in nuclear turnover or fibrosis were detected.

Key Words: ureter, vesico-ureteral reflux, hyaluronic acid, inflammation, fibrosis

Vesicoureteral reflux is a common and controversial problem in pediatric urology, affecting up to 1% of all children in the United States.[1] Traditionally patients with VUR underwent prolonged courses of antibiotic prophylaxis or open ureteral reimplantation. However, this dogma was recently challenged by the advent of newer endoscopic treatment methods and injectable materials.[2–4] Specifically the use of Dx/HA copolymer has greatly increased in this country in the last decade. Since its approval by the Food and Drug Administration in 2001, multiple series of Dx/HA use have been published, showing varying success rates.[4–7] Few problems or complications have been reported due to Dx/HA use.[8,9] However, relatively few studies have explored the histopathological effects of the implantation of Dx/HA in pediatric bladders.[10,11]

According to the United States Census Bureau the average American life span is now 77.6 years. Because injected Dx/HA is expected to remain in vivo for the remaining decades of a patient life, it would appear that the long-term effects of these substances are significantly under studied. Therefore, we present our experience with patients undergoing subureteral and/or intraureteral Dx/HA injection, and subsequent histopathological analysis.

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MATERIALS AND METHODS

Patient Selection and Clinical Features

Following approval by the institutional review board we reviewed the charts of all patients undergoing open reimplantation after failed Dx/HA injection(s) at a single pediatric institution. Each child undergoing Dx/HA injection at this institution is enrolled in a database maintained by study nurses and 1 of us (JCR). The database includes preoperative and postoperative voiding cystourethrogram results, age, gender, surgeon, injection technique, date of procedure(s), presence of dysfunctional voiding, neurogenic bladder, pre-injection surgical interventions, perioperative urinary tract infection(s), ureteral duplication anomalies, periureteral (Hutch) diverticula, amount of Dx/HA injected per ureter and postoperative complications, including obstruction. Followup voiding cystourethrogram and renal ultrasound are performed 3 months following injection. Cases are defined as failing injection therapy if they show no change bilaterally or a worse grade of reflux in either ureter on postoperative imaging.

At followup consultation after failed injection we discuss the available options with all patients and families. This includes observation with continued antibiotics, observation without continued antibiotics, repeat Dx/HA injection and open surgical reimplantation. Patients choosing this latter option were eligible for inclusion in this study.

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Pathological Features

Excised distal ureteral segments with periureteral tissues were examined by a single genitourinary pathologist (TJS). A histological and immunohistochemical battery of stains was used on each tissue block, including hematoxylin and eosin for routine morphology, CD3 for T lymphocytes, CD20 for B lymphocytes, MIB-1 as a marker for cell turnover and trichrome to assess for fibrosis. The pathological criteria evaluated were the presence, location and intensity of morphological abnormalities, fibrosis, GCR, chronic inflammation, eosinophilic infiltration, free Dx/HA, and CD3, CD20 and MIB-1 immunostaining. Free Dx/HA was defined as the presence of Dx/HA globules without a surrounding pseudocapsule of giant cells and fibrous tissue. CD3, CD20 and MIB-1 cell counts were defined semiquantitatively as the percent (in 5% intervals) of the total cell population present per high power field and qualitatively as none, focal, moderate or severe staining. Pathological features were then correlated with the time from injection to surgical excision. For patients undergoing more than 1 injection the date of the first injection was used for calculating implant duration.

Statistical Analysis

Statistical analysis was performed using SAS® software. Temporal relationships were evaluated using Spearman's correlation tests. All p values were 2-sided and due to the relatively small number of patients assessed $p < 0.10$ was considered statistically significant.

RESULTS

Clinical Results

Distal ureteral segments of 1 boy and 15 girls (total of 30 ureters) were examined. Mean patient age was 4.5 years (range 0.4 to 9.7). Median time from injection to implant excision was 8 months (range 3 to 22). VUR grade was unilateral grade I to bilateral grade IV with no patient having grade V VUR. Three ureters were grade I VUR, 8 were grade II, 14 were grade III and 5 were grade IV. Three patients had anatomical anomalies, including a Hutch diverticulum and complete ureteral duplication in 1 each, and the 2 conditions in the remaining patient. Dysfunctional voiding was present in 5 patients (31%). The mean injected volume of Dx/HA was 1.0 cc per ureter. Of the patients 14 (88%) underwent bilateral injection, 15 (94%) underwent 1 Dx/HA injection and the remaining patient received 2 injections before proceeding to open surgery. No patient experienced any perioperative complications as a result of Dx/HA implantation or ureteroneocystostomy. No clinical covariate (age, gender, VUR grade, amount of Dx/HA injected, voiding dysfunction or anatomical anomaly) correlated with the duration of Dx/HA implantation or histological findings.

Histological and Immunohistochemical Results

The most common histological finding, GCR, was present in 15 patients (94%) and it was located in the serosa in 13 (87%). Figure 1 shows a representative GCR. Free Dx/HA was noted in 4 patients (25%) and a pseudocapsule surrounded the Dx/HA microspheres in 12 (75%). Focal chronic inflammation was present on hematoxylin and eosin staining in 8 patients (50%), while moderate inflammation was present in only 1 (6%). No patient demonstrated severe inflammation on hematoxylin and eosin examination. No patients showed significant morphological atypia or eosinophilic infiltrate. None of the histological features listed correlated with the duration of Dx/HA implantation or with clinical covariates.

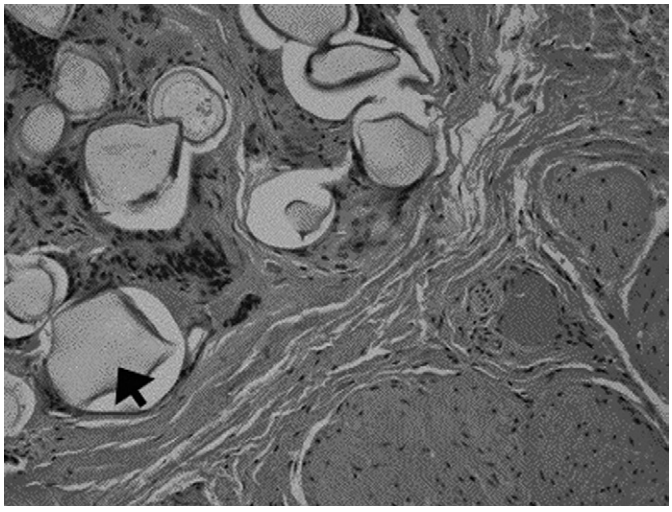

FIG. 1. Intense periureteral giant cell reaction encapsulating dextranomer microspheres (arrow). Reduced from ×200.

MIB-1 staining was present in 0% to 20% of cells per patient. Only 1 patient demonstrated no staining throughout all slides reviewed and the remaining patients showed focal to moderate staining. MIB-1 staining did not correlate with the duration of implantation ($p = 0.6$). As assessed by trichrome staining, fibrosis was present in all specimens and in 40% to 80% of each excised specimen (fig. 2). Fibrosis levels did not appear to change with the duration of implantation ($p = 0.8$). CD3+ T cells and CD20+ B cells were present in all patients at the ureteral lumen (fig. 3). The mean CD3+ T-cell count was 14% (range 1% to 50%) and the mean CD20+ B-cell count was 2.9% (range 0% to 10%). The 2 parameters ranged from no staining to moderate staining with no patient showing a strong lymphocyte presence. CD3+ and CD20+ lymphocyte counts increased concurrent with time from injection ($p = 0.06$ and 0.02, respectively).

DISCUSSION

In 1995 Stenberg and Lackgren introduced their experience with Dx/HA in animal models and in 75 children, reporting a 68% cure rate for grades III–IV VUR at 3 months.[12] In 2001 Dx/HA was approved by the United States Food and Drug Administration for use in children with primary grades I–IV VUR. Multiple series since that time have shown good to excellent success rates of 64% to 92% depending on the injection technique used and the population studied.[4–7] Indeed, an increasing number of clinicians are now choosing primary endoscopic management of VUR in children over the more traditional strategies of antibiotic prophylaxis or ureteroneocystostomy.[2,3]

It is reasonable to assume that children with VUR undergoing Dx/HA injection should have a normal projected life span, which is currently estimated to be 77.6 years in the United States.[13] The average age at injection is reported to be about 6 years,[14] which implies that injected Dx/HA should remain in vivo more than 7 decades. Clearly it is incumbent on pediatric urologists to prove the long-term