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Original Article

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Inflammatory Changes Induced by Dextranomer/Hyaluronic Acid Injection into the Several Different Wall Layers of Ureterovesical Junction

Deniz Mubaris Abdullahoğlu[1], Oktay Ulusoy[1], Osman Zeki Karakuş[1], Oğuz Ateş[1], Seda Özbal[2], Bekir Ergür[2], Gülce Hakgüder[1], Mustafa Olguner[1], Feza Miraç Akgür[1]

[1]Department of Pediatric Surgery, Dokuz Eylul University School of Medicine, Izmir, Turkey
[2]Department of Histology and Embryology, Dokuz Eylul University School of Medicine, Izmir, Turkey

ABSTRACT

Objective: Endoscopic subureteric injection (ESI) is a minimally invasive and effective method for the treatment of vesicoureteral reflux. Ureterovesical junction (UVJ) obstruction after subureteric dextranomer/hyaluronic acid (Dx/HA) injection has been reported in the literature. Inflammatory changes in the several different wall layers of the UVJ after injection of Dx/HA have not been investigated before. The aim of this study is to evaluate the inflammatory response induced by Dx/HA in the several different wall layers of UVJ in an experimental rat model.

Methods: Dx/HA was injected into the UVJ submucosally, intramuscularly, and adventitially to the consecutive group of rats. Inflammatory cell densities were determined and granuloma thickness was measured under the light microscope.

Results: Density of mononuclear cells, macrophages, giant cells, and thickness of granuloma formation have been found to be increased in the muscular layer and adventitial layer injection groups compared to the submucosal layer injection group ($P < .01$). However, the highest chronic inflammatory response has been found in the adventitial layer injection group whereas the lowest chronic inflammatory response has been found in the submucosal layer injection group ($P < .01$).

Conclusion: We conclude that injection of the Dx/HA into the correct ureteral wall layer is important to minimize the risk of severe inflammatory response resulting in UVJ obstruction.

Keywords: Reflux, obstruction, injection, experimental, inflammatory

Corresponding author: Oktay Ulusoy ✉ oktay.ulusoy@deu.edu.tr

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INTRODUCTION

Vesicoureteral reflux (VUR) is defined as a retrograde flow of urine from the urinary bladder up to the ureter and/or into the collecting system of the kidneys. VUR affects 1-2% of all children, and approximately one-third of children with VUR will experience urinary tract infection.[1] Endoscopic subureteric injection (ESI) is a minimally invasive and effective method for the treatment of vesicoureteral reflux. One potential complication after ESI is the development of ureterovesical junction (UVJ) obstruction. Dextranomer/hyaluronic acid (Dx/HA) is the current popular, safe and effective ESI material.[2-4] However, UVJ obstruction after ESI with Dx/HA has been reported in the literature and Dx/HA-induced inflammatory changes have been accused of UVJ obstruction.[5-7] Dx/HA-induced inflammatory changes in the several different wall layers of the UVJ have not been investigated before. We hypothesized that injecting Dx/HA into the different wall layers of UVJ may induce severe inflammation, which leads to UVJ obstruction. The aim of the present study is to evaluate the inflammatory response induced by Dx/HA into the several different wall layers of UVJ in an experimental rat model.

MATERIALS AND METHODS

Thirty-six adult (3-6 months) male Wistar albino rats (260-300 g) were used in the current study. A preliminary study was performed on one male Wistar albino rat to evaluate the applicability of the surgical technique. Intraperitoneal ketamine (60 mg/kg) plus xylazine

(5 mg/kg) was used for anesthesia. In this study, 0.05 mL Dx/HA was injected with a PPD syringe needle through the midline laparotomy under sterile conditions to the submucosal layer, muscular layer, and adventitial layer separately. Micro-surgical tools and a 10× magnifying operation microscope (OPMI-99, Carl-Zeiss, Jena, Germany) were used during vesicostomy and injection. The PPD syringe needle was marked for each layer. The rat was killed with cervical dislocation under ether anesthesia. The bladder was excised for histopathological confirmation. In the histopathological examination of the bladder, Dx/HA copolymer was found into the target location in each of the 3 histological layers. Subsequent injections were made with a marked PPD syringe needle.

The same surgical method was used in all rats except the control group. All injection volumes were identical, that is, 0.05 mL. Rats were housed in separate cages with controlled temperature and 12 h light/dark cycle. Postoperative analgesia was provided with meloxicam (1 mg/kg) for 2 days.

There were 5 experimental groups;

Control group (n = 7): No intervention group.
Sham (n = 7): Saline was injected into the submucosal layer of the left UVJ.
Submucosal injection (n = 7): Dx/HA was injected into the submucosal layer of the left UVJ.
Intramuscular injection (n = 7): Dx/HA was injected into the muscular layer of the left UVJ.
Adventitial injection (n = 7): Dx/HA was injected into the adventitial layer of the left UVJ.

Histopathological Analysis

Thirty days after the surgery, rats were killed with cervical dislocation under ether anesthesia. The bladders were totally excised and fixed in 10% formaldehyde for 3 days. The specimens were embedded in paraffin and 5 µm serial sections (Rotary microtome, RM 2155, Leica, Germany) were obtained. The slides were deparaffinized in xylene. Hematoxylin and eosin staining and Masson's trichrome staining were used for histopathologic evaluation.

Five sample sites (each one with a surface area of 200 μm^2 per microscopic field) were selected randomly to count the density of inflammatory cells and to measure granuloma thickness under the light microscope (Olympus BX-51, Tokyo, Japan). The tissue images were obtained (Olympus DP-71, Tokyo, Japan) and evaluated with computerized micro-camera analysis (UTHSC (The University of Tennessee Health Science Center) image software). Mast cell, eosinophil, mononuclear cell, macrophage, and giant cell densities were evaluated. Data were expressed as mean ± 1SD.

Statistical analyses were performed using SPSS software version 22.0. Kruskal–Wallis analysis of variance was used to compare results among groups and Mann–Whitney *U* test was performed to test the significance of pairwise differences using Bonferroni's correction for multiple comparisons.

Table 1. Densities of Mast Cells and Eosinophils (mean ± 1SD)

Groups	Mast Cells	Eosinophils
Control	0.09 ± 0.10	0.06 ± 0.09
Sham	0.08 ± 0.08	0.09 ± 0.29
Submucosal injection	0.17 ± 0.24	0.14 ± 0.22
Intramuscular injection	0.26 ± 0.22	0.20 ± 0.16
Adventitial injection	0.29 ± 0.10	0.14 ± 0.15

RESULTS

No perioperative or postoperative death was encountered. The densities of mast cells and eosinophils were similar among groups (Table 1).

Regarding the densities of mononuclear cells and macrophages, no statistically significant differences were found between control (Figure 1) and sham groups (Figure 2). The densities of mononuclear cells and macrophages were found significantly increased in submucosal injection (Figure 3), intramuscular injection (Figure 4), and adventitial injection groups (Figure 5) compared with the control group ($P < .01$). The densities of mononuclear cells and macrophages were found to be lowest in the submucosal injection group and highest in the adventitial injection group (Table 2).

No giant cell and granuloma formation was detected in the control and sham groups. The density of giant cells and granuloma thicknesses of the intramuscular injection group was found to be significantly increased compared with submucosal injection group ($P < .01$). The density of giant cells and granuloma thickness were found to be increased in the adventitial

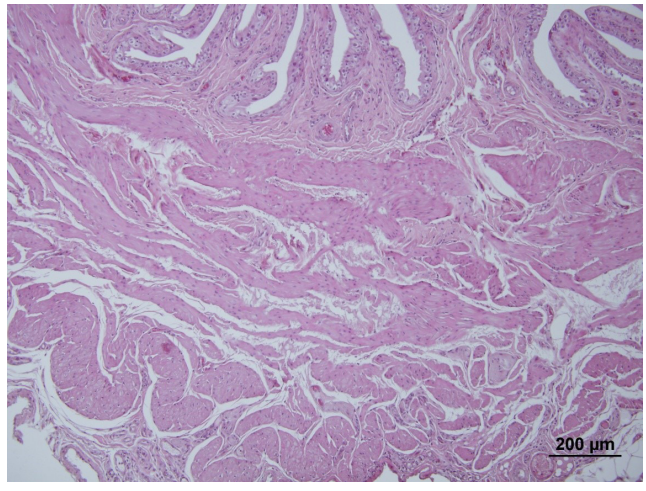

Figure 1. The histopathological image of the control group.

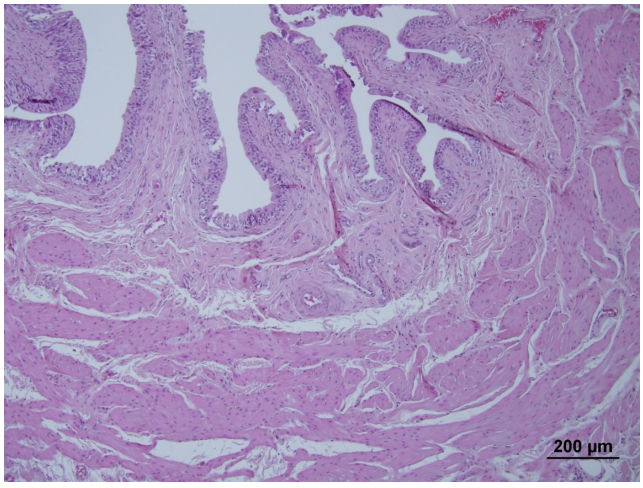

Figure 2. The histopathological image of the sham group.

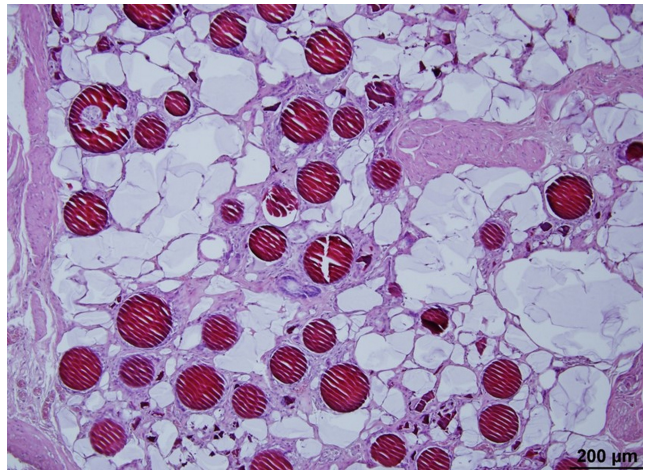

Figure 4. Dx-HA injection into the muscular layer.

injection group compared with the intramuscular injection group ($P < .01$). The density of giant cells and granuloma thickness were found least in the submucosal injection group and highest in the adventitial injection group (Table 3).

DISCUSSION

In the last decades, ESI has become the first choice for the treatment of VUR.[3-8] Various complications have been reported after ESI with different injection materials.[5-7,9] Polyacrylate polyalcohol copolymer (PPC) (Vantris®, Promedon, Cordoba, Argentina), which is a non-biodegradable material, has been used for ESI since 2008 and low VUR recurrence rate has been reported in the literature.[10] However, UVJ obstruction has been reported after ESI with PPC.[11] Experimentally, it has been shown that PPC causes severe inflammatory reaction and fibrosis more than biodegradable materials.[12] Authors have suggested that severe inflammatory reaction plays a role in UVJ obstruction after ESI with PPC.[12] Popular biodegradable materials are non-animal stabilized Dx/HA copolymer (Deflux®; Q-Med Scandinavia Inc., Uppsala, Sweden) and cross-linked sodium salt Dx/HA (Dexell®, Eva-Tech, Lyon, France). Although granuloma and collagen formation due to biodegradable materials are less than non-biodegradable materials,[12] early or delayed UVJ obstructions have been reported after ESI with Dx/HA.[5-7,13] Early UVJ obstructions have occurred between the first few days and 3 months after ESI with Dx/HA.[13-16] It is possible that both temporary edema and bulking effect of Dx/HA lead to the early transient obstruction that is seen 0.7%.[17] However, UVJ obstruction has completely resolved after temporary ureteral stent placement without open surgery in all patients.[17]

Delayed UVJ obstruction has been reported after ESI with Dx/HA.[5-7,13] Dysmorphic nature of the ureter orifice, congenital refluxing megaureter with a distal aperistaltic segment (beak sign),[18] excessive volume injection, comorbid diagnosis with VUR or severe, and progressive inflammatory foreign body reaction after ESI with Dx/HA have been accused regarding delayed

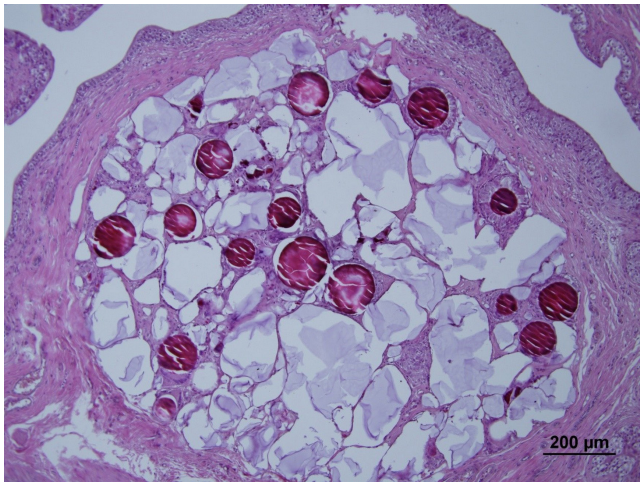

Figure 3. Dx-HA injection into the submucosal layer.

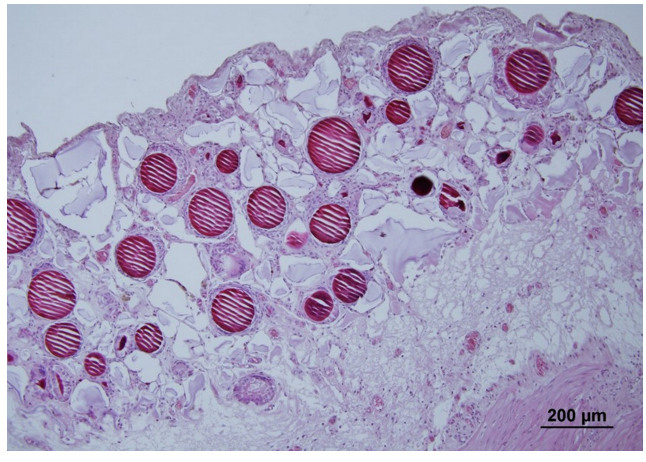

Figure 5. Dx-HA injection into the Adventitial layer.

Table 2. Densities of Mononuclear Cells and Macrophages (mean ± 1SD)

Groups	Mononuclear Cells	Macrophages
Control	0.23 ± 0.60	0.09 ± 0.15
Sham	0.29 ± 0.75	0.14 ± 0.29
Submucosal injection	7.29 ± 3.02*	2.00 ± 0.53*
Intramuscular injection	16.31 ± 2.79*†	5.70 ± 0.84*†
Adventitial injection	30.71 ± 5.83*‡	8.20 ± 1.27*‡

*P < .001 compared with the control and sham groups. †P < .01 compared with the submucosal and adventitial injection groups. ‡P < .01 compared with the submucosal and intramuscular injection groups.

Table 3. Density of Giant Cells and Granuloma Thickness (Mean ± 1SD)

Groups	Giant Cells	Granuloma Thickness (µm)
Submucosal injection	0.37 ± 0.21	64.1 ± 4.00
Intramuscular injection	1.0 ± 0.26*	74.9 ± 1.46*
Adventitial injection	2.1 ± 0.39†	84.5 ± 1.66†

*P < .01 compared with the submucosal and adventitial injection groups. †P < .01 compared with the submucosal and intramuscular injection groups.

UVJ obstructions.[5-7,13,14,17,19,20] Arlen et al. noted that in 1 patient, progressive hydroureteronephrosis had been occurred by 0.7 mL Dx/HA injection to a single ureter, which they had to perform open ureteral re-implantation 15 months after ESI.[14] They observed the size of the Dx/HA beads at the ESI site was visually increased and the volume removed intraoperatively was larger than 0.7 mL. The histopathologic analysis of the distal ureter showed a foreign body giant cell reaction in adjacent layers of the ureteral wall.[14] Their results are important in that there may be a severe inflammatory response in different ureter wall layers induced by Dx/HA.[14] Rubenwolf et al. have shown granulomatous foreign body reaction in the histopathologic evaluation of excised ureteric segments in delayed UVJ obstruction.[5] They hypothesized that the injection material may have been implanted into the adventitial layer of UVJ.[5] Hypothesis of Rubenwolf et al. and Arlen et al. may be correct.

In our clinical experience, we have experienced UVJ obstructions requiring ureteral re-implantation after Dx/HA injection in 2 patients (8th and 13th months after ESI with Dx/HA) and we have found our overall rate of this complication as 0.4% (2 in 501 ureters) (unpublished data). The preoperative voiding cystourethrography showed grade 3 and 4 VUR on the right side in both patient and no appearance compatible with the distal aperistaltic segment (beak sign). Excessive peri-ureteral fibrotic tissue was observed macroscopically during open ureteral re-implantation surgery, especially in the adventitial layer. The histopathological evaluation of the excised UVJ segments showed a severe chronic inflammatory response, multinuclear giant cells, and granuloma formation. These results have shown similarities to the ureteral histopathological findings of reported delayed cases in the literature. This clinical experience has led us that implantation of the injection material into the ureteral adventitia during ESI or local migration of the injected material from the submucosal layer to the adjacent wall layers of the ureter may be the cause of UVJ obstruction secondary to peri-ureteral severe inflammation. In the current study, the density of mononuclear cells, macrophages, giant cells, and granuloma formation has been found to be increased in the muscular layer injection group compared with the submucosal layer injection group. However, the highest inflammatory response has been found in the adventitial layer injection group whereas the lowest inflammatory response has been found in the submucosal layer injection group. We think that showing the increased inflammatory response in several different wall layers of the UVJ is important in explaining particularly progressive delayed UVJ obstruction.

Neutrophils and mast cells moderate acute inflammatory response.[21] The acute inflammatory response caused by biomaterials usually resolves within 1 week. Following acute inflammation, the presence of mononuclear and giant cells is considered as the evidence for chronic inflammation.[22] The limit of the current study is that histopathological evaluation of the specimens was done 30 days after injection of the material. The 30 days interval is insufficient to determine whether the inflammation encountered around the microspheres of ESI material is self-limiting or progressive. In order to observe long-term effects of the ESI material, that is, whether chronic inflammation encountered is self-limiting or progressive; a post-injection time interval longer than 6 months is required. Another limitation of the current study is the fact that the deficiency of comparison of the intervened ureter to the contralateral side within the same rat.

As shown in this study, these different histopathological responses triggered by Dx/HA in different ureteral wall layers may be the cause of UVJ obstruction in the long term. Considering this information, it is important that ESI should not be performed outside the submucosal area in daily clinical practice. However, intraoperatively, if it has been detected that the injection material has been given outside the submucosal area for any reason, it has been vital that these patients be followed up more closely in the long term in terms of UVJ obstruction.

CONCLUSION

This study has shown that inflammatory cellular response and thickness of the granuloma formation correlate with the injection of the Dx/HA material to the different wall layers of UVJ. The thickness of the granuloma and inflammatory responses induced by Dx/HA is highest after adventitial layer injection and lowest after submucosal layer injection. We conclude that injection of the Dx/HA into the adventitial or muscular layer

of UVJ causes an increased inflammatory response. ESI with Dx/HA into the correct ureteral wall layer is important to minimize the risk of severe inflammatory response resulting in UVJ obstruction.

Ethics Committee Approval: Ethics committee approval was received from the Dokuz Eylul University Ethics Committee for Animal Research (Approval no: 63/2014).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflict of interest to declare.

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