Dextranomer/Hyaluronic Acid Copolymer (Dx/HA) Has no Effect on Bacterial Growth in Culture Media With or Without Antibiotic Discs

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ABSTRACT

Introduction: *Endoscopic treatment of vesicoureteral reflux (VUR) by subureteral injection of biocompatible polymers is an established treatment option for reflux. Dextranomer/hyaluronic acid copolymer(Dx/HA) has gained wide popularity for treating VUR. We decided to investigate the antibacterial activity of Dx/HA and its interaction with antibiotics in in-vitro conditions.* **Materials and Methods:** *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus mirabilis suspensions were inoculated into Mueller-Hinton agar media and 30 µl of Dx/HA was inoculated in 5 mm diameter pits and the plates were incubated at 37°C for 24 hours. At the end of the incubation period, inhibition zones around the discs were measured. Expansion of the inhibition zones towards the pits which contained Dx/HA was considered as synergism. Dx/HA was inoculated into pits made in Mueller-Hinton agar medium without antibiotic discs but containing suspensions of bacteria. These media were incubated under the same circumstances and same measurements were done. All experimental procedures were performed twice. Increase in bacterial zone diameters for ≥ 5 mm was inoculated was regarded as significant for each agent.* **Results:** *Dx/HA caused no difference in bacterial growth either with or without antibiotic discs as determined by inhibition zones in the culture media.* **Conclusion :** *Dx/ha will not contribute to UTI if it is used for the treatment of VUR in cases either with or without infection.*

KEYWORDS: Vesicoureteric reflux, deflux, urinary tract infection

INTRODUCTION

Vesicoureteric reflux (VUR) is a common urinary tract anomaly, affecting approximately 0.4-1.8% of healthy children, and is present in approximately 30-50% of children diagnosed with a febrile urinary tract infection (UTI).[1] VUR allows the retrograde flow of urine from the urinary bladder into the upper parts of the urinary tract. Its combination with UTI and pyelonephritic scarring can lead to severe changes to the kidneys and to the development of so called reflux nephropathy. The possibility of spontaneous regression of reflux has enabled medical therapy to find a place in the treatment of VUR. Antibiotic prophylaxis and management of bladder dysfunction have achieved excellent results in keeping low grade VUR.[2] However, it requires long-term antibiotic management and this is likely to induce resistance.[1]

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Prevention of the sequelae of febrile UTI is the goal of VUR management. Although ureteral reimplantation has long been the gold standard for surgical treatment of VUR, it has been associated with a febrile UTI rate of 25- 40% in successfully treated patients.[3] In all cases that are in between, endoscopic treatment is the treatment of choice. It represents an alternative to long term antibiotic therapy and to open surgical treatment.

The endoscopic administration of bulking agents has now gained wide popularity and is becoming an effective alternative, even in severe VUR. The main reasons for this success have been its minimal operative stress, and high cure and low complication rates. In contrast to open surgery, endoscopic treatment has been demonstrated to be associated with a much lower postoperative incidence of UTI. Elder et al, [4] reported an overall cystitis rate of 6% and febrile UTI rate of 0.75% following endoscopic treatment using a multitude of agents. Lackgren et al,[5] initially reported their febrile UTI rate to be 8% during a 5-years follow up. More recently Stenberg and Lackgren6 reported their incidence of UTI to be 25%; however, only 3.4% had documented febrile UTIs in the 7-12 years following endoscopic treatment.

Currently, 88% of surgical interventions for VUR are performed endoscopically. Open ureteral reimplantation is reserved for patients who have failed endoscopic treatment, high-grade VUR, megaureters and parental preference.[1] In recent years, a new absorbable bulking agent, dextranomer/hyaluronic acid copolymer(Dx/HA) (Deflux™, Q-MED AB, Uppsala, Sweden), has gained wide popularity for treating VUR.7 So we intended to investigate this widely used material called Deflux regarding its antibacterial activity and interaction with antibiotics. As this investigation is difficult to perform on humans and needs a large number of individuals to be enrolled in the study, we decided to carry out the research under in-vitro circumstances. To this end, the interaction between Dx/HA and the most frequently involved microorganisms in childhood urinary infections and utilized antibiotics were investigated.

MATERIALS AND METHODS

To investigate the interaction between Dx/HA and the most frequently involved microorganisms in childhood UTI and utilized antibiotics, we prefered an in-vitro growth-medium. Standard strains of Escherichia coli (E. coli) ATCC 25922, Pseudomonas aeruginosa (P. aeruginosa) ATCC 27853 and Klebsiella pneumoniae (K. pneumoniae) and Proteus mirabilis (P. mirabilis) strains isolated from patients were cultured for 24 h's. Antibiotic discs (Bioanalyse®, Turkey) absorbed with amikacin (AK, 30 µg), ampicillin/sulbactam (SAM, 10/10 µg), imipenem (IPM,10µg), trimethoprim/ sulphamethoxazole (SXT, 1.25/23.75 µg), cefepime (FEP, 30 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), gentamicin (CN, 10 µg), and cefoperazone/ sulbactam (CES, 75/10 µg) were used. Bacterial suspensions of 0.5 McFarland turbidity were prepared and inoculated onto Mueller-Hinton agar medium. To identify the antibacterial efficieny of Dx/HA and to asses synergistic or antagonistic effect that could exist with antibiotics, a method modified from Manchanda and Singh was used.[8] Pits with 5 mm diameter were made at 3 cm distance from the antibiotic discs in these plates. Thirty µl of Dx/HA was inoculated into these pits and the plates were left for incubation at 37°C for 24 h's. At the end of the incubation period, inhibition zones that formed around the discs were evaluated. Expansion of the inhibition zones towards the pits which contained Dx/HA was interpreted as synergism. To investigate the effect of Dx/HA independently of antibiotics, Dx/HA was inoculated into pits made in Mueller-Hinton agar medium without antibiotic discs but inoculated with the bacterial suspensions. After these growth medium were incubated under the same circumstances they were evaluated. All tests were performed twice. Increase in zone diameter (≥ 5 mm) in which plates containing inoculated Dx/HA was regarded as significant for each agent.[8]

RESULTS

The inhibition zones around the antibiotic discs were the same statistically either with addition of the Dx/HA or without it for each type of the bacteria used (Table I, $p>0.05$ for each comparison). When used alone without any antibiotic discs, Dx/HA caused neither larger inhibition zones nor more bacterial proliferation around the pits compared with bacterial inoculation alone, for each of type of the bacteria.

Antibiotics	*K. pneumoniae*		*E. coli*		*P. aeruginosa*		*P. mirabilis*	
	Dx/HA (+)	Dx/HA (-)	Dx/HA (+)	Dx/HA (-)	Dx/HA (+)	Dx/HA (-)	Dx/HA (+)	Dx/HA (-)
FEP	22,2	22,3	30,3	30,2	Resistant	Resistant	28,1	28,2
IPM	30,1	30,0	30,0	30,1	27,1	27,0	30,3	30,2
AMC	25,0	25,1	21,2	21,3	Resistant	Resistant	28,2	28,3
CN	17,2	17,3	23,5	23,4	17,3	17,4	23,1	23,0
CES	31,2	31,1	33,2	33,1	28,4	28,3	26,6	26,7
SAM	24,1	24,0	21,3	21,4	Resistant	Resistant	22,4	22,3
SXT	Resistant	Resistant	12,0	12,1	Resistant	Resistant	Resistant	Resistant
AK	26,2	26,3	25,3	25,4	25,2	25,3	26,2	26,1

DISCUSSION

Dx/HA is a recently developed organic substance consisting of microspheres with diameters from 80 to 250 µm. These constituents form a viscous biodegradable solution that is non-allergenic, non-mutagenic and nonimmunogenic. The injected Dx/HA volume decreases slightly, and ingrowth of fibroblasts and generation of collagen between the microspheres may account for the endogenous tissue augmentation and a smaller loss of volume than expected.[2] There is controversy regarding association of Dx/HA injection with UTI rates. In a study, Dx/HA injection has been demonstrated to be associated with a much lower postoperative incidence of UTI. Cystitis and febrile UTI rates were 6% and 0.75% following endoscopic subureteric injections.[4] On the other hand, in one randomized trial comparing antimicrobial prophylaxis with Dx/HA injection, children in the Dx/HA group actually had more UTIs than those in the antimicrobial prophylaxis group (6/40 versus 0/21, respectively) during the 12-month follow-up period.[9]

This pioneering study was planned to evaluate the effect of interaction of the material Dx/HA with frequently used antibiotics on bacterial growth and to understand whether it has an antibacterial effect at the injection site or a colonization promoting property. As this study is inconvenient to perform on humans and requires plenty of subjects, we decided to investigate Dx/HA in in-vitro circumstances. In the present study, the most frequently encountered microorganisms responsible for UTI in children, namely, E. coli, P. aeruginosa, K. pneumoniae and P. mirabilis and the most commonly utilized antibiotics in the treatment of UTI namely amikacin, ampicillin/sulbactam, imipenem, trimethoprim/ sulphometoxazole, cefepim, amoxicillin/ clavulanic acid, gentamicin and cefoperazone/ sulbactam were used to evaluate the interaction with Dx/HA. This investigation revealed that there was neither an interaction between Dx/HA and microorganisms nor Dx/HA and the commonly used antibiotics. That is, Dx/HA neither supports

nor inhibitis bacterial growth when used alone or in combination with the antibiotics. These results support the view that the sole endoscopic injection of Dx/HA has no effect on UTI.

In the light of these results, we propose that, under appropriate antiseptic cystoscopic intervention circumstances- Dx/HA can be administered to UTI cases under antibiotic treatment or UTI cases with antibiotic resistant microorganisms which are accompanied by VUR. But this consideration requires further studies.

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