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先天性單側腎盂輸尿管阻塞狹窄處之免疫發炎機制之探討

與研究

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Role of the immuno-inflammatory reaction in stricture site of congenital unilateral ureteropelvic junction obstruction (2/2)

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Abstract

Background/Purpose: Ureteropelvic junction (UPJ) obstruction is defined as a functional or anatomic obstruction to urine flow from the renal pelvis to the ureter that left untreated results in symptoms or renal damage. The neonatal kidney is more susceptible to injury than the adult kidney following ureteral obstruction, such that growth and function of the developing kidney are impaired to a greater extent. Design an animal model that will present as partial obstruction to narrow the ureter diameter and then we speculate that urothelium could be injury due to the Banuli effect. We speculate the consequential effect of immuno-inflammatory response and then finally to the reaction of muscular hypertrophy or lamina propria with collagen deposition.

Methods: Forty 2 months old rabbits were used for the experiment. Under general anesthesia, an 0.5mm polyethylene tubing 50 catheter were placed to surround and compass the ureteropelvic junction. Histology of renal and stenotic ureter segment were investigated and observation.

Results: Increases in the intrapelvic pressure, dilated pelvic area, and thinning of cortical thickness were noted and exacerbated during the follow-up. Obvious mononuclear cells infiltration were also found in the renal parenchymal area. Dilated tubules with atrophic tubular cell and interstitial fibrosis were noted, too. However, proliferation or hypertrophy of smooth muscle cells were not apparent within the stenotic segment of ureter.

Key words: human, child, kidney pelvis, ureteral obstruction, inflammatory cell
Introduction

In the pediatric population, congenital urinary tract obstruction is the most common fetal anomaly identified in antenatal screening of pregnant women, with an incidence of up to 1% of all pregnancies. However, it may be defined as a functional or anatomic obstruction to urine flow from the renal pelvis to the ureter that left untreated results in symptoms or renal damage. Renal failure can result from the effects of urinary tract obstruction to delay growth and maturation of the developing kidney. The neonatal kidney is more susceptible to injury than the adult kidney following unilateral ureteral obstruction (UUO), such that growth and function of the developing kidney are impaired to a greater extent. The clinical disease entity of ureteropelvic junction obstruction (UPJ-O) may not be a static lesion but rather a dynamic and evolving process. Flashner SC et al. had demonstrated that nonobstructive hydronephrosis, diagnosed prenatally, might later convert to obstruction at the UPJ or in the juxtavesical ureter (UVJ). Till now pathogenetic processes of UPJ-O may not be a static lesion but rather a dynamic and evolving process. The obstruction may change temporally, becoming diminished over time, progressive, or occurring intermittently. Flashner SC et al. had demonstrated that nonobstructive hydronephrosis, diagnosed prenatally, might later convert to obstruction at the UPJ or in the juxtavesical ureter (UVJ).

Material and methods

Inclusion criteria for patients

All procedures involving animals were approved by the local Institutional Animal Care and Use Committee. White New Zealand rabbits (mean 1.5 kg) were obtained from animal laboratory of NCKU medical center and housed with free access to food and water. Rabbits were fully anesthetized with phenobarbital (250 mg/kg intravenously) and bilateral kidney was prepared as described previously, using modifications of established methods. Briefly, each ureter was immediately exposed by laparotomy and dissection and then surrounded and compassed with polyethylene tubing 50 catheter. After this, each rabbit was sacrificed according to the scheduled time point (one week, two weeks, 4 weeks, and 8 weeks). During these processes, the dissected kidneys and the stenotic ureters were processed for further investigations.

Routine histology and Immunohistochemistry for tissue cytokines and chemokines

All stenotic ureters and renal specimens were obtained at the time of dismembered pyeloplasty and fixed in 10% buffered formalin. Additionally, eight UPJ-O segment specimens were immediately snap frozen in Tissue-Tek
optimum cutting temperature compound (Sakura Finetek, Torrance, CA, USA). Routine histological hematoxylin and eosin (H&E, Sigma-Aldrich, St. Louis, MO, USA) stains were prepared for each specimen.

Immunoreactive target proteins were detected by polyclonal or monoclonal antibodies with the avidin-biotin immunoperoxidase method (Ultratech HRP streptavidin-biotin detection kit; Immunotech, Marseille Cedex, France), according to the manufacturer’s instructions, overnight at 4°C in a humidified chamber. Diaminobenzidine (DAB) or 3-amino-9-ethylcarbazole (AEC; DAKO) was used as a chromogen and counterstained with methylene green (Sigma-Aldrich) or hematoxylin (Sigma-Aldrich).

**Measurement procedures and methods**

Positive and negative control slides for IHC and ISH were processed simultaneously by using the relatively corresponding positive controls. The negative control group included ureter segment tissues and renal specimens from five received shame operated rabbit and not received operated rabbit. Pathological evaluation of the UPJ specimens was based on the H&E stains and examined targets using a grading modified from that of Zhang et al. For quantitative analysis of the immunohistochemical findings, we adopted the method for the morphometric analyses (18-20) and the tissue images were captured with Image-Pro plus software (ver. 4.51; Media Cybernetics, Silver Springs, MD, USA). The density of each dot in the image was indicated by digits according to a 36-bit color scale in which white=0 and black=4095. Each slide with an IHC finding was evaluated for foci per non-overlapping microscopic findings of 10 high-power fields and integrated optical density ($\times10^{6}$) was calculated for grades of severity. We counted nuclear Ki67 stains as a percentage of positive cellular staining. Mast cell tryptase was evaluated as the number of total mast cells and activated mast cells appearing as size swelling and/or degranulation for foci per non-overlapping microscopic finding of 10 high-power fields. The negative control group was processed as above. A negative result would indicate the absence of antibody staining in non-stenotic UPJ segment tissue or tissue below the stenotic UPJ-O segment. For ISH, however, the findings were compared between the study and control groups only.

**Statistical analysis**

Statistical analysis was done with a commercially available computer software package (StatView 5.01; Abacus Concepts, Inc., Berkeley, CA, USA). Unless indicated otherwise, continuous data were expressed as mean ± standard error of the mean (SEM). When two groups were compared, the Mann-Whitney test or chi-square test, depending on whether the data were continuous or categorical, was performed initially to confirm the presence of significant differences.

**Results**

Forty 2 months old New-Zealand rabbits, 20 male and 20 female, were enrolled in the study. They were arranged to 4 groups (1w, 2w, 4w, and 8 w) and each group contained with 5 rabbits that received PE50 tubing. In briefly, our results were showed in 2 figures as below.

Figure 1A and B: PE 50 tube was compassed and surrounded proximal ureter to induce UPJ-obstruction.

Figure 1C and D: renal parenchymal tissue showed glomerular congestion, tubular dilatation, tubular atrophy, interstitial space increasing, and inflammatory cell infiltration in 2w and 4w after obstruction of ureter.

Figure 1E to 1H: they showed the grossly morphologic change of each kidney that after obstruction of proximal
ureter. According to our finding, it demonstrated that enlarged kidney could be induced after obstruction of ureter and the effect would exacerbate from 1w to 4w. Besides, shrinkage of renal size, that might due to renal parenchymal loss was noted in 8w after obstruction of ureter.

Figure 2A to 2D: it showed the histological pattern of stenotic ureter segment. In this area, we just found mild urothelium hyperplasia in 8w group rabbit only. Submucosa showed with increased collagen deposit, that combined with infiltration of little inflammatory cells.

Figure 2E to 2H: it showed the coronal section of non-obstructed kidney with obstructed side kidney. Apparently dilated pelvicalyceal area was noted in obstructed kidney and it combined with parenchymal tissue loss. Obviously, contralateral kidney showed quite normal.

Discussion
The role of epithelium in the pathogenesis of respiratory or gastrointestinal diseases is understood. However, it has not received much attention in genitourinary diseases, especially obstructive uropathy. In this study, we demonstrated that an urothelium abnormality in was present in the tissue of obstructed ureteropelvic junctions, which was first described by Bartoli et al. In our previous study also agreed with their opinions. For further investigations, we explored the distribution of all different inflammatory cells.

In summary, inflammatory network occurred in human local UPJ-O segment and interaction could be noted between inflammatory and urothelium. Renal parenchymal insult in our research model is the same as other research model. However, our results of this rabbit model couldn’t support the role of cytokine or chemokine responses in urothelium as a mucosal cytokine network, which leads to the mononuclear cell and eosinophil infiltration. For these reasons, we speculate that rabbit or method of this external compassion is not fit to the research of urothelial-mesenchymal trophic mechanism. Further investigation of the immunopathological features and consequences of the inflammation in UPJ-O should be looked for.

Acknowledgments
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References


14. Stadnyk AW: Intestinal epithelial cells as a source of inflammatory
cytokines and chemokines. *Can J Gastroenterol* 16:241-246, 2002


**Figure legend**

Figure 1. Grossly and histological demonstration of this rabbit UPJ-O animal experimental model.

Figure 2. Grossly and histological demonstration of stenotic ureter segment and renal morphology in this rabbit UPJ-O animal experimental model.