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

計畫類別：個別型計畫
計畫編號：
執行期間：93年08月01日至94年07月31日
執行單位：國立成功大學醫學系小兒科

計畫主持人：邱元佑
共同主持人：謝式洲
計畫參與人員：林秀冠

報告類型：精簡報告
處理方式：本計畫可公開查詢

中華民國94年07月01日
Abstract

Background  The cytokine-producing ability of urothelium, a urinary tract barrier between urine and underlying connective tissue, may play a role in exacerbating the pathogenesis of congenital ureteropelvic junction obstruction (UPJ-O) disease. The potential role of urothelium in human urinary tract obstruction has been described in our previous study. In this study, we investigated the immunopathological cellular marker in children with congenital UPJ-O and evaluated their distributions.

Methods  Twenty children with congenital UPJ-O who had received pyeloplasty were enrolled. Morphological abnormalities as well as pathological and inflammatory changes of UPJ-O segments were studied. The expression of cellular marker and chemokines in urothelium were investigated and compared with control tissue by immunohistochemistry (IHC).

Results  Variable degrees of inflammatory cell and IL10 were characteristic findings then control non-obstructed tissue (P<0.05). CD3 and CD8 were expressed both in urothelium, lamina propria, and muscularis propria. Augmented expression of IL-10 is also detected by IHC and was observed in the urothelium of UPJ-O segments. However, CD4 was quite lesser than CD8. Significantly higher levels of ST2L or CXCR3 were noted within urothelium.

Conclusions  This study demonstrated that urothelium, like intestinal and respiratory epithelia, plays an active role in the immunoregulatory process, and possibly contributes to the exacerbation of the pathogenesis of congenital UPJ-O. Many These immunopathological reactions within urothelium may underlie the pathogenesis of UPJ-O.

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 (UO), 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. 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). Till now pathogenetic processes of UPJ obstruction had been classified as: 1) intrinsic stenosis/valves; 2) insertional anomaly; 3) fibrous bands/adhesion and 4) aberrant crossing vessels. However, Bartoli et al. hypothesized that primary urothelium breaks with urine spread inside the ureteral wall and the subsequent activation of leukocytes might be the primary event that lead to UPJ obstruction. In animals, urothelium hyperplasia was found in experimental hydronephrosis and pyelonephritis. To our knowledge, changes of urothelium had not been mentioned in UPJ-O before. These data indicate the association between urothelial aberration and UPJ-O. It has also been reported that epithelial cells adjacent to transitional cell carcinoma may undergo hyperplastic change due to paracrine regulation by tumor-derived growth factors, raising the possibility that abnormal local production of growth factors may be the cause of urothelial hyperplasia. For this reason, we speculate that the initial event would occur at the break of urothelium and then in situ production of cytokines or chemokines will consequently attract the infiltration of inflammatory cells. Then, after the interaction of cytokines/growth factors and mesenchymal cell (smooth muscle cell), results of smooth muscle hypertrophy or interrupted muscularis propria will occur and lead to the clinical UPJ obstruction.
Material and methods

Inclusion criteria for patients

This prospective study was designed for all children who were evaluated for hydronephrosis and were admitted to our medical center during the 5-year period ending December 31, 2004. Children with vesico-ureteric junction obstruction, vesico-ureteric reflux, infravesical obstruction, or renal dysplasia were excluded from the study. Patients with congenital unilateral UPJ-O confirmed by an antegrade pyelogram and who had received dismembered pyeloplasty were included. The function of the obstructed side kidney was evaluated by 99m-technetium diethylenetriaminepentaacetic acid (DTPA). Renal sonography showed normal size and contour for the contralateral kidneys of all patients. Surgical indications were symptomatic presentation, thinning cortical thickness, and/or grade 4 hydronephrosis by renal echography, and a clearance half-time (T (1/2)) of DTPA > 20 min. Our institutional review board approved the study protocol. The objectives and details of our study were explained to the parents and to patients old enough to understand. Parental approval was obtained before performing pyeloplasty. According to the renal function of the initial DTPA, children with results in the obstructed-side kidney > 40% were classified as group I (good function), and those with a result ≤ 40% were group II (poor function) as from our previous study. A renal biopsy was performed to assess the progression of renal disease.

Routine histology and Immunohistochemistry for tissue cytokines and chemokines

All UPJ-O 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. Additionally, IHC was performed to standard procedures for:

1. CD3: total T lymphocyte
2. CD4: T helper cell
3. CD8: T suppressor cell
4. CD19: B lymphocyte with plasma cell
5. CD20: B lymphocyte without plasma cell
6. CD25: as IL-2 receptor alpha chain. When co-expressed in CD4+ T cells (CD4+CD25+ T cells), it is defined as regulatory cells and as a functionally mature T cell subpopulation. Their functions are able to inhibit the function of T cells carrying the same T-cell receptor specificity and prevent rejection in an antigen-specific, dose-dependent manner.
7. CD45RA: isoform of CD45
8. CD45RA: isoform of CD45, T-cell subset (express on resting CD4 T cells)
9. CXCR3(CXC-chemokines Receptor 3): as the marker for Th1 cell (induces chemotactic migration in inflammation-associated effector T cells)
10. ST2L: as a cell surface marker which has been
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 following positive controls: tonsils of chronic tonsillitis for IFN-γ, TNF-α and turbinate mucosa of allergic rhinitis for IL-4, IL-5, eotaxin, and mast cells. The negative control group included five UPJ segment tissues from children with Wilms' tumor and four frozen ureter tissue segments from below the UPJ-O segments of patients. In addition, ISH without the target cDNA probes was performed for the negative control groups.

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.12. 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 (×10⁶) 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. Paired ELISA data between the UPJ-obstructed-side kidney and the contralateral normal-side were analyzed using the Wilcoxon signed rank test. Statistically significant differences between groups were defined as \( P<0.05 \) and are indicated in the text.
Results

Twenty children, 12 boys (age range, 1 to 120 months; mean, 13.3 months; median, 1.5 months) and 8 girls (age range, 4 to 180 months; mean, 71.0 months; median, 62.5 months), were enrolled in the study. Eight children presented with abdominal pain. Five of the seven children presenting with a urinary tract infection (UTI) had been prenatally diagnosed with hydronephrosis. Although 15 infants had been suspected to have UPJ-O based on prenatal sonography, only 14 (12 male and 2 female) of them were diagnosed at or before 6 months of age with UPJ-O.

Twenty-four patients underwent a DTPA before they had pyeloplasty. According to the radionuclide renal function test on the obstructed side, 15 patients (11 boys, 4 girls; age: 21.8±9.7 months; median: 4.0 months) were placed in group I (DTPA: 48.3±1.1%) and 9 patients (5 boys, 4 girls; age: 50.4±22.5 months; median: 5.0 months) were in group II (29.2±2.3%).

Twenty tissue segments of obstructed UPJs were collected and the characteristics of pathologic presentation were analyzed.

*Urothelium hyperplasia with infiltration of different cellular marker in UPJ-O tissue*

Pathological findings of the UPJ-O specimens demonstrated a marked epithelial cell thickening in our subjects since the transitional urothelium frequently exceeded six layers. In suffered patients, each cellular marker of inflammatory cells could express in situ. In general, prominent CD3 positive cells infiltrated not only in urothelium, but also in lamina propria and smooth muscle (figure 1A), CD4 positive cells (figure 1B) are fewer than CD8 cells and they were only lighter expressed in lamina propria. However, CD8 cells (figure 1D) were infiltrated more quite even in local tissue of urothelium and other connective tissues. Mature B cells that expressed as CD20 were also in submucosa connective and urothelium (figure 1C). For this reason, we further investigated the expression of T helper 1 (CXCR3 positive cell) and T helper 2 cell (ST2L positive cell). To exceed our expectations, basal layer of urothelium could express the cellular characters of T helper 1 cells (figure 1F). But CXCR3 (figure 1E) was lesser in urothelium and also within lamina propria of smooth muscle. Regulation of T cells was examined as the expression of CD45RO (figure 1H) and cytokine IL10 (figure 1G), and these were also positive expression in urothelium infiltrated mononuclear cell (IL10) and lamina propria (CD45RO).

For further investigations, we compared the significant difference between UPJ-O patient and control patient. In briefly, all cellular marker that we described previously were all elevated in UPJ-O patients (Figure 2B, 2C). In particular, more CD3 expressed tissue would expressed more CD4 and CD8 and always little expression of CD20 cells.
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 this local UPJ-O segment and interaction could be noted between inflammatory and urothelium. Our results 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 can speculate that an urothelial-mesenchymal trophic unit would become active to drive pathologic remodeling and smooth muscle proliferation through complex cytokine interactions. Further investigation of the immunopathological features and consequences of the inflammation in UPJ-O should be valuable.

Mucosal surfaces are sites of antigenic exclusion, antigenic sampling, and immune regulation. Our study demonstrated that urothelial cells, similar to intestinal and respiratory epithelial cells, might have an active role in the immunoregulatory process and may contribute to the exacerbation of congenital UPJ-O. We cannot exclude that alternative explanations include the possibility that urinary obstruction or any type of tissue damage secondary to urinary obstruction may be the initiating event that accounts for all the observable data. Nevertheless, based on our findings, we may speculate that in the initial primary urothelium break stage of UPJ-O, chemokines produced by the urothelium may be the chemoattractants for leukocytes. These events may lead to further inflammatory responses such as inflammatory cell infiltration, mast cell migration, and activation. Likewise, the combined effects of the urothelium and leukocytes may establish a local cytokine environment and lead to a self-amplifying process typical of chronic inflammatory tissue. Detailed knowledge of the cytokine responses in the urothelium is necessary for a better understanding of the role of urothelial cellular reaction in congenital UPJ-O. Our findings may provide the basis for early intervention to prevent congenital hydronephrosis in the future.

Acknowledgments
This project was funded in part by grants NSC 89-2314-B-006-024 from the National Science Council of the Republic of China and No. 046, 2001, from the Scientific Funds of the National Cheng Kung University Medical Center.
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. Distribution of different cellular markers of inflammatory cells in UPJ-O segments.

Figure 2. Distribution of different cellular markers of inflammatory cells in different patients or between UPJ-O and non-obstruction control tissues.
Figure 2

A

- CD3
- CD20

Count/X200HPF

0.0  2.5  5.0  7.5  10.0  12.5  15.0  17.5

Patients

- CD4
- CD8

Count/X200HPF

B

CD3  CD4  CD6  CD20  CD3  CD4  CD6  CD20

UPJ-O ureter  Non-obstructed ureter

C

CD45RA  CD45RO  IL-10

CD25  CD45RA  CD45RO  IL-10

UPJ-O ureter  Non-obstructed ureter