Abstract. – OBJECTIVE: The purpose of this investigation is to determine if the protein expression of the Wnt/β-catenin signal pathway is induced after acute renal injury in patients with acute tubular necrosis.

PATIENTS AND METHODS: Sixty patients in whom met inclusion criteria underwent renal biopsy. Based on the result of the biopsy, patients were divided into two groups: one with nephrotic syndrome with mini-change disease (MCD group, 30 patients), the other one with minor lesions and acute tubular necrosis (ATN, 30 patients). Biopsy sections were stained with either periodic acid-Schiff (PAS) or Masson stain and examined under light microscopy to determine the degree of tubulointerstitial injury and renal tubular interstitial fibrosis, respectively. The expression of Wnt/β-catenin signal proteins in renal tissue was resolved immunohistochemically using protein-specific antisera.

RESULTS: The typically silent Wnt/β-catenin signal pathway in normal kidneys was noted to be upregulated in patients in either the MCD and ATN groups; that expression was statistically significantly higher in the ATN group as compared to the MCD group ($p < 0.05$). The degree of renal tissue injury was statistically significantly higher in the ATN group as compared to the MCD group ($p < 0.05$). Also, the degree of tubulointerstitial fibrosis was significantly higher in the ATN group as compared to the MCD group ($p < 0.05$).

CONCLUSIONS: Results of this investigation confirm the expression of Wnt/β-catenin signal pathway in renal tissue in patients with acute renal injury and nephrotic syndrome, which appear to be important in the host biological response to necrosis, fibrosis, and proliferation.

Key Words: Acute tubular necrosis, Renal micro-lesion, Wnt/β-catenin signaling pathway

Introduction

Acute kidney injury is a sudden episode (within a few hours or a few days) of kidney failure or damage that can result from many different causes including decreased renal blood flow, direct damage to the kidneys (e.g., cancer, inflammation or infection) or blockage of the urinary tract. Acute kidney injury can be reversed. However, if the offending cause is not corrected, permanent damage can occur leading to chronic kidney disease that can progress to end-stage renal disease$^{1,2}$. The pathogenesis of acute kidney injury and potential treatment strategies are under investigation. Recent studies have demonstrated that activation of the Wnt/β-catenin signaling pathway plays a role in the pathogenesis of kidney injury caused by chronic obstructive nephropathy and renal interstitial fibrosis$^3$.

It has become clear that the Wnt/β-catenin signaling pathways are important for both kidney development and the pathogenesis of renal disease. The Wnt signaling protein family plays an important role in the renal development. However, these gene products are silent in mature kidneys$. The primary mechanism of renal tubular injury is due to renal tubular contracture that creates changes in hemorheology resulting in tissue ischemia and hypoxia. During injury, inflammatory factors can also contribute to renal tubular injury; the Wnt signaling proteins are known to have a role in an inflammatory mediator response. Although most of the renal damage from acute injury can be self-repaired, about 18 to 30% of patients with acute kidney injury will not be fully restored to their normal state and develop either chronic kidney disease or end-stage renal disease. Treatments for acute renal failure are important in order to slow the progression to chronic renal disease and to improve the patient’s prognosis. Through a better understanding of the molecular mechanisms underlying the pathogenesis of acute kidney injury novel treatment strategies can be developed to improve clinical outcomes$^{5,8}$. 

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Wnt comprises a family of proteins secreted by endocrine organs that have signal pathway conduction functions. The protein family can activate target cell by interacting with membrane receptors and promote the development of tissues and organs. There are various members of the Wnt protein family that participate in the developmental process. In early kidney development, Wnt4 and Wnt9b have a very high expression that affects the structure and function of kidney development. In the mature kidney, the Wnt signaling pathway is silent, but it can be activated after renal injury. Abnormal Wnt protein secretion can induce renal cysts. In the Wnt7b knockout mice model, it was found that in an ischemia-reperfusion acute renal injury model, loss of renal function was irreversible and tissue regeneration repair was inhibited.

The purpose of this study is to investigate the relationship between Wnt/β-catenin signaling pathway in acute kidney injury and renal tubular injury repair in nephrotic syndrome patients with biopsy-confirmed mini-change disease (MCD group) or with minor lesions and acute tubular necrosis (ATN).

Patients and Methods

Sixty patients with nephrotic syndrome were enrolled in the study. All patients underwent renal biopsy and were then divided into 2 groups; one with small focal lesion (30 patients with MCD) and the other with acute tubular necrosis (30 patients with ATN). The day before the biopsy, blood and urine samples were collected for measurement of blood biochemical parameters (serum creatinine, blood lipids, blood glucose, serum albumin) and renal function (24-hour urinary protein quantitation). Before the biopsy, all patients fasted for at least 10 hours. The study was approved by the Ethics Committee of Huaihe Hospital of Henan University and informed consents were signed by the patients and/or guardians.

Biopsy samples were fixed in a neutral formaldehyde fixative solution, embedded in paraffin, and then sectioned into 3 mm thick slices. Sections were stained with periodic acid-Schiff (PAS) or Masson stain and examined under light microscopy (600 × magnification) to determine the degree of tubulointerstitial injury and renal tubular interstitial fibrosis, respectively. Tubulointerstitial injury scoring was also performed from analysis of histological section; the scoring included the following factors: renal tubular dilatation, tubular atrophy, interstitial fibrosis and cell infiltration (the scoring method is shown in Table I). The degree of renal tubular interstitial fibrosis was determined as the percentage of a positive area covering the renal parenchyma from photographs of the cortex and outer medullary region of samples by image analysis (Motic Med 6.0A digital medical image analysis system). Immunostaining was performed to determine the qualitative protein levels of Wnt4 protein or β-catenin protein expression. Briefly, immunostaining was performed using an immunohistochemistry kit provided by Beijing BoAosen Biotechnology Co., Ltd. (Beijing, China) (batch number: SP-0022) according to the manufactures protocol using β-actin antibody (1:5,000 Invitrogen, Carlsbad, CA, USA) or Wnt antibody (Ser-33/Ser-37/Thr-41; 1:300, Cell Signaling Technology, Danvers, MA, USA).

Statistical Analysis

Data were analyzed using SPSS30.0 statistical software (Version X; IBM, Armonk, NY, USA). The t-test was used for the measurement data and the χ²-test was used for the count data. The statistical significance was set at p < 0.05.

Results

Clinical blood biochemical parameters of the two groups were statistically analyzed; there were no significant differences between patients with either MCD or ATN (p > 0.05) (Table II).

Table I. Tubulointerstitial injury scoring.

<table>
<thead>
<tr>
<th>Levels</th>
<th>Score</th>
<th>Renal tubular dilatation</th>
<th>Tubular atrophy</th>
<th>Interstitial fibrosis</th>
<th>Cell infiltration</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>/N</td>
<td>/N</td>
<td>/N</td>
<td>/N</td>
</tr>
<tr>
<td>Local lesions</td>
<td>1</td>
<td>&lt; 25%</td>
<td>&lt; 25%</td>
<td>&lt; 25%</td>
<td>&lt; 25%</td>
</tr>
<tr>
<td>Multiple lesions</td>
<td>2</td>
<td>26%-50%</td>
<td>26%</td>
<td>50%</td>
<td>26%</td>
</tr>
<tr>
<td>Large lesions</td>
<td>3</td>
<td>50%-75%</td>
<td>75%</td>
<td>75%</td>
<td>50%</td>
</tr>
<tr>
<td>Diffuse lesions</td>
<td>4</td>
<td>&gt; 75%</td>
<td>&gt; 75%</td>
<td>&gt; 75%</td>
<td>&gt; 75%</td>
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Expression of the Wnt/β-catenin signal pathway in patients with acute renal injury

The degree of tubulointerstitial injury (determined from PAS staining: Figure 1 are representative histomicrographs and Figure 2 represents the mean and standard deviation of the percentage of positive PAS cells) and tubulointerstitial injury score (Table III) was significantly higher in the ATN group as compared to the MCD group (p < 0.05).

The qualitative Wnt/β-catenin protein expression in the renal tissue of the two groups was resolved by immunohistochemically staining. The percentage of the positive area was calculated by the image analyzer (Motic Med 6.0A Digital Medical Image Analysis System). The expression levels of Wnt/β-catenin in ATN group was statistically significantly higher than MCD group (p < 0.05) (Figure 3 are representative histomicrographs and Figure 2 represents the mean and standard deviation of the percentage of positive Wnt and β-catenin cells).

Masson staining was performed to determine the amount of tubulointerstitial fibrosis in renal tissue of both groups. All the cortical and extra-medullary sections were photographed under 600 × magnification using a light microscope. The percentage of a positive area covering an area of renal parenchyma in each image was calculated by image analysis to evaluate the levels of interstitial fibrosis in each patient with a renal injury. The results showed that the percentage of positive Masson staining in the ATN group (Figure 4A) was significantly higher than the MCD group (Figure 4B), and that difference was statistically significant (p < 0.05) (Figure 4C).

![50 µm](A)

![50 µm](B)

**Figure 1.** PSA staining (renal cortex and medulla presented a positive reaction with purple color): [A] ATN group [B] MCD group. (Magnification 100 ×).
Discussion

Many patients with an acute kidney injury will not fully recover, despite treatment; many of those patients will have their renal function deteriorate to chronic kidney disease or end-stage renal disease1. The prognosis for patients with either chronic kidney disease or end-stage renal disease is poor. The Wnt protein family bind to a cell membrane receptor to regulate the growth and development of organs by either autocrine or paracrine secretion. In the mature

Table III. Renal interstitium score (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Renal interstitium score</th>
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<tbody>
<tr>
<td>MCD (n=30)</td>
<td>9.37 ± 1.35</td>
</tr>
<tr>
<td>MCD and ATN (n = 30)</td>
<td>14.47 ± 2.13</td>
</tr>
<tr>
<td>t-value</td>
<td>21.7</td>
</tr>
<tr>
<td>p-value</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure 2. Blind analyses on paraffin sections by PAS staining (small grid counted representing tubulointerstitial injury). The numbers of small grids with tubulointerstitial injury performance were counted in order to calculate the percentage of positive staining cells. The staining of ATN group was statistically significantly greater than the MCD group (p < 0.05).

Figure 3. Immunostaining of renal biopsy samples for Wnt/β-catenin: [A] MCD group, [B] ATN group; Wnt/β-catenin were expressed in both groups; [C] Compared with MCD group, the expression of Wnt/β-catenin was statistically significantly increased in ATN group (p < 0.05) (Magnification 100 ×).
Expression of the Wnt/β-catenin signal pathway in patients with acute renal injury

human kidney, Wnt signaling is usually silent\textsuperscript{11,13}. In this investigation, the Wnt/β-catenin signaling system was activated in patients with acute renal injury, suggesting an attempted at repair. It is possible that further upregulation of the Wnt/β-catenin signaling system could enhance the repair and/or completely reverse the damage that occurs after renal injury. In a study of Wnt/β-catenin signal modulators, it was reported that repair of renal tubular injury is very likely to be targeting the renal tubular endothelium, which could mitigate the occurrence chronic kidney disease after acute renal injury. If this premise is correct, then the Wnt/β-catenin could improve the cure rate after renal injury. Results from this investigation showed the expression of Wnt/β-catenin protein in both MCD and ATN patients; the qualitative expression was significantly higher in the ATN patients than in the MCD patients ($p < 0.05$). PAS staining also indicated that there were variable levels of renal tissue injury in both MCD and ATN patients; the level of injury was significantly higher in ATN patients ($p < 0.05$). In addition, there was evidence of tubulointerstitial fibrosis observed by Masson staining in both MCD and ATN patients; the amount of fibrosis was statistically significantly higher in the ATN patients ($p < 0.05$). In ATN patients, renal interstitial tissue, including the renal medulla and the medulla

Figure 4. Masson staining of renal tissue biopsies to determine the degree of fibrosis: representative histomicrographs (A) ATN group (B) MCD group. The percentage of positive Masson staining cells in ATN group was statistically significantly higher than MCD group (C) ($p < 0.05$) (Magnification 100×).
oblongata, had acute necrosis and fibrosis due to the acute ischemic injury, which is consistent with the pathologic outcome from ATN. The results showed that the proliferation of tubular epithelial cells was significantly increased in ATN patients compared with MCD patients ($p < 0.05$). This suggests that Wnt/β-catenin system may be involved in the proliferation of tissue and that it is activated in the necrotic kidney tissue after ATN.

Wnt/β-catenin signal pathway and the Wnt4 protein expression are upregulated after renal tubular endothelial cell injury. However, there are no definitive studies to prove that Wnt4 protein is an independent risk factor for renal tubular endothelial cell injury or that other types of Wnt protein ligands might also be involved in renal tubular endothelial cell injury. At present, the activation of Wnt/β-catenin signaling pathway has been identified to play a critical role in the acute renal failure, and this study also demonstrates that the upregulation of this pathway is related to the renal tubular endothelial cell injury. In the future study of acute renal injury, the role of the Wnt/β-catenin signal pathway and other non-classical Wnt signaling pathways of β-catenin independent should be studied to determine which are involved in injury recovery to provide a basis for novel treatment strategies after renal injury in order to improve patient prognosis.

**Conclusions**

Strengths of this investigation are the study of patients with confirmed MCD or ATN by clinical renal biopsy and the comparison of the expression level of Wnt/β-catenin signaling protein between the two groups as the host response to injury (e.g., fibrosis and proliferation). Since the Wnt/β-catenin signaling pathways appear to play a role in human renal injury, it may also be a novel target for the clinical treatment of patients with an acute renal injury. Further studies on the role of Wnt pathway plays during acute renal injury are needed in order to clarify the pathogenesis of acute renal injury and find new therapeutic targets.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

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