Effect of Tolvaptan in Patients With Chronic Kidney Disease Due to Diabetic Nephropathy With Heart Failure

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SUMMARY

The efficacy of tolvaptan for treating heart failure has already been shown. Adequate data relating to the effect of tolvaptan on the correlation of water balance in renal disease are not available. A retrospective study was conducted on the efficacy and adverse reactions of tolvaptan for treating nephrotic syndrome.

The subjects were 26 patients with chronic kidney failure due to diabetic nephropathy with heart failure who were administered tolvaptan and seen between December 2011 and October 2013. The endpoints were urinary output, physical findings, and blood analyses. The expression of aquaporin-2 in the collecting duct, which is related to the action of tolvaptan, was investigated by immunohistochemistry using the kidney tissue obtained for the diagnosis.

Responses were seen in 19 of the patients. In the histopathological investigation there was severe glomerulosclerosis in patients with diabetic nephropathy, but the responders were noticeable in that they only had mild tubulointerstitial damage. Non-responders exhibited profound tubulointerstitial damage. The expression of aquaporin-2 was determined in 8 patients, of which 7 were responders who tested positive for aquaporin-2. The remaining case was a non-responder who showed no expression of aquaporin-2.

Tolvaptan is considered effective for some cases of nephrotic syndrome. There are no clear parameters for predicting an effect, but the present study showed that aquaporin-2 was expressed in the epithelial cells of the collecting ducts of tolvaptan responders. (Int Heart J 2014; 55: 533-538)

Key words: Histopathological findings, Aquaporin-2

Tolvaptan is a diuretic with a new mechanism that produces water diuresis by selectively binding to the vasopressin V2-receptors, and disturbs the movement of aquaporin-2 into the luminal side of collecting duct cells through activation of cAMP.1,2 The efficacy of this drug for treating heart failure has already been shown.3,4 In particular, there have been a number of case reports for treating fluid retention in congestive heart failure where there is insufficient response to treatment using existing diuretics such as furosemide.5 Tolvaptan is a new water diuretic that inhibits water reabsorption in the kidney collecting ducts and elicits a diuretic effect without electrolyte output.6 It has already been demonstrated that existing Na diuretics are effective against intractable chronic heart failure. Loop diuretics, which are typified by Na diuretics, have a strong diuretic action, but they may also exacerbate renal function through electrolyte imbalance or by activating the renin-angiotensin-aldosterone system (RAAS), which causes concern for the impact of these drugs on the prognosis of patients.7 It is known that in heart failure secretion of vasopressin is promoted through enhancement of the RAAS, but tolvaptan is considered to be a diuretic that matches the pathology of heart failure.8 The EVEREST study and ACTIV in CHF trial reported this drug had short-term and mid-term effects in cases of CHF in the United States and Europe.8,9

Conversely, adequate data are not available related to the effect of tolvaptan in cases of chronic kidney disease (CKD). Some believe that the effect of tolvaptan cannot be anticipated for complicated cases of renal dysfunction based on the action of this drug in the kidneys, but some studies showed that tolvaptan may be effective for volume control and diuretic-induced hyponatremia in CKD patients.10 Furthermore, we have experienced the clinical effect of promoting diuresis through administration of tolvaptan to patients with existing diuretic-resistant heart failure and severe renal dysfunction equating to approximately eGFR 10-20 mL/minute/1.73m², which resulted in avoiding dialysis therapy. However, the mechanism of action of tolvaptan in kidney failure pathology remains unknown. The main property of tolvaptan is a diuretic action in the renal collecting ducts via aquaporin-2, and while it is thought that the expression of aquaporin-2 affects the pharmaceutical effect, this aspect has not yet been investigated.

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In this study a retrospective investigation was conducted into the efficacy and adverse drug reactions of tolvaptan in the treatment of diabetic nephropathy with heart failure and in patients for whom general diuretic monotherapy had proved ineffective. Other aims were to investigate various specific parameters and histopathological findings in responders and non-responders, as well as investigating the involvement of aquaporin-2 using kidney tissue. In addition, the effect of tolvaptan in patients with renal failure will be discussed, as well as cases where tolvaptan would be indicated.

**METHODS**

The subjects included 26 patients with chronic kidney disease due to diabetic nephropathy with heart failure who were administered tolvaptan and seen in the hospital between December 2011 and October 2013. Tolvaptan was administered as a single oral dose of 7.5-15 mg per day depending on the case. By systemic state, water intake was free on a case-by-case basis. The endpoints investigated were urinary output after administration, physical findings (presence/absence of edema or pulmonary edema), and blood analyses [serum creatinine (Cre), eGFR, serum sodium (Na), serum BNP (BNP), and serum albumin (Alb)] before and/or after administration of tolvaptan. We defined effective cases as those showing a 2-fold increase in urinary volume compared to pre-administration levels or a clear improvement in edema or heart failure symptoms. Cases where the urinary output did not satisfy the aforementioned criteria, cases with exacerbated edema and/or heart failure, and cases that were introduced to dialysis therapy were deemed to be non-responders.

We evaluated and scored kidney biopsy specimens for tubulointerstitial lesions in 8 cases who underwent kidney biopsy. We evaluated the degree of interstitial fibrosis and tubular atrophy as a percentage of the total involved area in the interstitium and tubules. A score of 0 was assigned when the biopsy specimen showed no interstitial fibrosis and tubular atrophy; a score of 1 was assigned when less than 25% was affected by interstitial fibrosis and tubular atrophy; a score of 2 was assigned when at least 25% but less than 50% of the biopsy specimen had interstitial fibrosis and tubular atrophy; and finally, a score of 3 was assigned when at least 50% of the biopsy specimens was affected by interstitial fibrosis and tubular atrophy was present.

Immunohistochemistry was also used to examine aquaporin-2 expression in the epithelial cells of the collecting ducts relating to the mechanism of tolvaptan action and its relationship to the efficacy of tolvaptan.

Aquaporin-2 immunohistochemistry method (polymer technique): The tissue obtained through kidney biopsy and normal control kidney tissue from a nephrectomy case were fixed in 20% neutral buffered formalin, embedded in paraffin, and sections of 2-3 μm thickness were prepared. Slides with the sections were deparaffinized, immersed in purified water, and then autoclaved using an antigen activation solution at pH 9. After washing with TBS for 5 minutes × 3 times, endogenous peroxidase was blocked by washing in 3% hydrogen peroxide in methanol for 10 minutes, and then washed with TBS for 5 minutes × 3 times. The primary antibody was used after determining the optimal dilution. The slides were incubated with antibody at room temperature for 1 hour, and washed with TBS for 5 minutes × 3 times, after which we determined the enzymatic/secondary antigen labeling polymer reagent response.

The Simple Stain MAX-PO (MULTI) (NICHIREI BIO-SCIENCES INC.) Polymer reagent instillation was performed as follows: room temperature for 30 minutes and then washed with TBS for 5 minutes × 3 times. After this, the samples underwent color development with adjusted DAB substrate solution. The reagent on the slide was washed for a few minutes with purified water, and then immersed in purified water. The specimens were finally stained with hematoxylin as a contrast agent, and then washed for 2 minutes, the color enhanced, the specimens dehydrated, penetrated and mounted, after which the specimens were observed using a light microscope. The primary antibody and dilution rate were anti-aquaporin-2 antibody (NB110-74682, 1:200, Novus Biologicals). The results were scored as follows; a score of (-) was assigned when the collecting duct showed no staining; a score of (+) was assigned when less than 25% was stained; a score of (+++) was assigned when at least 25% but less than 50% was stained, and a score of (++++) was assigned when at least 50% of the biopsy specimens was stained as described in a previous report.

**RESULTS**

The background and efficacy results of the 26 cases receiving tolvaptan are shown in the Table. Each of the endpoints was investigated. In comparing the test results between responders and non-responders for diabetic nephropathy, no difference was noted in the serum Cre, eGFR, and Alb levels. However, after tolvaptan was administered, serum Na and BNP levels showed a significant difference in responders, but not in non-responders.

In the responder group, the test results before and after administration for diabetic nephropathy showed the serum BNP level was significantly reduced from 459.0 ± 409.1 pg/mL to 206.4 ± 194.9 pg/mL (P = 0.04), while the serum Na level was significantly increased from 138.9 ± 3.7 mEq/L to 141.7 ± 3.0 mEq/L (P = 0.002).

A pathologist blinded to the eGFR results scored the biopsy specimens for interstitial fibrosis and tubular atrophy. We compared the eGFR values before administration of tolvaptan to the score of interstitial fibrosis and tubular atrophy. As a result, the average eGFR was 40.9 mL/minute/1.73m² in patients with a score of 0, 36.6 mL/minute/1.73m² in those with a score of 1, 4.4 mL/minute/1.73m² in those with a score of 2, and 29.0 mL/minute/1.73m² in those with a score of 3. We did not observe a clear association between the eGFR and the degree of tubulointerstitial lesions.

The histopathology of kidney biopsies from responders and non-responder was studied. The findings of a responder case are shown in Figure 1. This diabetic nephropathy patient had severe glomerulosclerosis and exudative lesions. There was also mild to moderate tubular atrophy and interstitial fibrosis. The biopsy specimen of a non-responder with chronic kidney failure caused by diabetic nephropathy is shown in Figure 2. This patient had severe glomerulosclerosis and exudative lesions and severe tubular atrophy and interstitial fibrosis were
Table. Clinical Parameters of Responders and Non-Responders in Diabetic Nephropathy (26 cases)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Responders (19)</th>
<th>Non-Responders (7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.6 ± 13.0</td>
<td>68.9 ± 14.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Gender (Male)</td>
<td>13</td>
<td>4</td>
<td>0.59</td>
</tr>
<tr>
<td>Pre Urine Volume (mL)</td>
<td>702.7 ± 509.4</td>
<td>1090 ± 365.1</td>
<td>0.23</td>
</tr>
<tr>
<td>Post Urine Volume (mL)</td>
<td>2616.7 ± 1273.0</td>
<td>900 ± 500</td>
<td>0.03</td>
</tr>
<tr>
<td>Pre NYHA class</td>
<td>2.7 ± 0.9</td>
<td>2.6 ± 1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Post NYHA class</td>
<td>1.4 ± 0.5</td>
<td>2.7 ± 1.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Pre dose of Furosemide (mg)</td>
<td>56.3 ± 41.3</td>
<td>68.6 ± 30.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Post dose of Furosemide (mg)</td>
<td>52.1 ± 43.3</td>
<td>74.3 ± 27.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Pre eGFR mL/minute/1.73m²</td>
<td>22.1 ± 18.1</td>
<td>13.6 ± 7.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Post eGFR mL/minute/1.73m²</td>
<td>21.5 ± 18.5</td>
<td>12.0 ± 8.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Pre Alb g/dL</td>
<td>3.0 ± 0.4</td>
<td>2.8 ± 0.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Pre Na mEq/L</td>
<td>138.9 ± 3.7</td>
<td>137.6 ± 4.6</td>
<td>0.43</td>
</tr>
<tr>
<td>Post Na mEq/L</td>
<td>141.7 ± 3.0</td>
<td>137.3 ± 6.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Pre BNP pg/mL</td>
<td>459.0 ± 409.1</td>
<td>734.6 ± 749.0</td>
<td>0.41</td>
</tr>
<tr>
<td>Post BNP pg/mL</td>
<td>206.4 ± 194.9</td>
<td>510.7 ± 338.3</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*P value difference of pre and post urine volume, †P value difference of pre and post NYHA class (I: score 1, II: score 2, III: score 3, IV: score 4), ‡P value difference of pre and post dose of furosemide, §P value difference of pre and post serum creatinine, #P value difference of pre and post eGFR, ||P value difference of pre and post serum sodium, ¶P value difference of pre and post serum BNP.

Figure 1. Kidney biopsy showing histopathological findings in a responder. The kidney biopsy specimen from a 40-year-old man with chronic kidney disease caused by diabetic nephropathy is shown. This patient had severe glomerulosclerosis and exudative lesions. There was also evidence of mild to moderate tubular atrophy and interstitial fibrosis (A, B). Tolvaptan responders with diabetic nephropathy showing positive staining for aquaporin-2. The positive staining was localized to the apical membranes in epithelial cells of the collecting tubules (C, D).
also present. Tamm-Horsfall protein (THP) was also noted. The average score was 0.86 (0-2) in the responders group (7 cases) among those with diabetic nephropathy; on the other hand, the score was 3 in the one non-responder. Although it was difficult to make a rating in such a small number of cases, the trend of a low score in responders was seen from the data obtained.

Aquaporin-2 expression was studied in a total of 8 patients who underwent kidney biopsies. All of the tolvaptan responders with diabetic nephropathy showed positive reactions (+: 2 specimens, ++: 1, +++: 4) for aquaporin-2 (Figures 1). The non-responder was negative (-) for aquaporin-2 (Figure 2). Aquaporin-2 expression was localized to the apical membranes of epithelial cells in the collecting tubules. Aquaporin-2 expression was absent in non-responder. Aquaporin-2 was not expressed in the glomerulus, the kidney tubules, or the interstitial tissue.

**DISCUSSION**

This was a retrospective study on the effect of tolvaptan in patients with diabetic nephropathy with heart failure. Treatment was deemed effective in 19 of the 26 cases. An increase in urinary output was achieved even in patients with severe kidney dysfunction. Also, in conditions such as hypoalbuminemia presenting with diabetic nephropathy where little effect is anticipated with diuretics, there were some cases where tolvaptan was effective. While there were only a small number of such cases, it suggests the idea of a new therapeutic option.

In the investigation of the clinical data, changes were noted in urinary osmolality in existing reports on heart failure patients, and the patients were categorized into responders and non-responders. We also compared serum and urinary osmolality before and after administration of tolvaptan, but there was no difference between responders and non-responders (data not shown). This could be due to the subjects already having nephropathy and the urinary concentration was already being decreased prior to administration of tolvaptan.

Body weight loss is a key element in the decision on effectiveness. However, in the present study we examined it in only 11 cases with recorded changes in body weight because body weight could not be determined in some patients with heart failure and breathlessness. Also, body weight could not be measured accurately in some patients due to worsening of the general condition or removal of water by starting hemodialysis therapy. The average body weight loss in the responder group was 11.1 kg after administration of tolvaptan.

Adverse reactions to tolvaptan are reported to include elevated serum Na levels. This could be seen as a result of generating diuresis, but none of the patients in this study had stopped administration or experienced adverse reactions, such as altered mentation, due to hypernatremia induced by administration of tolvaptan. However, after administration of tolvaptan there was a significant rise in serum Na levels in the responder group compared to the non-responder group. This was considered a result of the diuresis induced by tolvaptan.

Regarding kidney function, serum Cre and eGFR levels...
pre- and post-administration were not different in the 2 groups. There were 2 patients in whom tolvaptan was effective despite their eGFR levels being in the 5 mL/minute/1.73m² range. Thus, it is not always possible to predict the effect of tolvaptan based on serum Cre and eGFR levels. The histopathological results of the responders with diabetic nephropathy who had undergone a kidney biopsy showed severe glomerular lesions, relatively mild tubulointerstitial damage, and positivity for aquaporin-2 expression. This suggests that even in cases where there is severe nephropathy with high serum Cre levels, tubulointerstitial function may be maintained to a certain extent, particularly in the collecting ducts of the medullary area.

The histopathological findings are unclear due to the small number of subjects. However, in the kidney biopsies taken from patients with diabetic nephropathy, when there was no or only mild tubulointerstitial damage (fibrosis, inflammatory cell infiltration), tolvaptan was effective even when glomerulosclerosis was present. Conversely, in tolvaptan non-responders with diabetic nephropathy there were tissue findings that showed tubular atrophy and severe interstitial cell infiltration with THP casts occupying the tubule lumen. This suggests that the efficacy of tolvaptan may be affected by the extent of tubulointerstitial damage. In other words, it was shown that the efficacy of tolvaptan may be weakened in patients with more severe tubulointerstitial damage than in patients with glomerular changes.

Aquaporin-2 is attracting attention as a potential therapeutic target for water balance disorders which commonly occur in many diseases. Brown, et al reported that vasopressin plays a role in regulating water and acid-base balance by stimulating accumulation of the aquaporin-2 water channel in the apical membranes of collecting duct principal cells by an immunostaining method. Kwon, et al showed the localization of aquaporin-2 by immunofluorescence and immunoelectron microscopy. We conducted immunohistochemistry tests to investigate the expression of aquaporin-2 in the epithelial cells of the collecting tubules to further investigate the efficacy of tolvaptan. In existing reports the expression of aquaporin-2 in kidney tissue has been examined in a variety of kidney diseases, and these reports indicate that the expression is reduced in kidney disease compared to normal kidneys. In our study, we investigated the relationship between the expression of aquaporin-2 and the efficacy of tolvaptan. We found, as mentioned above, that there was a greater number of responders who were positive for aquaporin-2 expression.

As far as we were able to ascertain, there has been little published on the efficacy of tolvaptan and the expression of aquaporin-2 in kidney tissue. Biomarkers that would indicate whether the effect of tolvaptan can be expected are still at the investigative stage. A previous paper demonstrated that responses to tolvaptan were significantly associated with elevated urine aquaporin-2 levels. We plan to investigate the influence of urinary aquaporin-2 and the expression of aquaporin-2 on the efficacy of tolvaptan in future studies.

The efficacy of tolvaptan is not reliant on the expression of aquaporin-2 alone. Another factor may be AVP resistance of selective down-regulation of the V2 receptor. It has been reported that the expression of aquaporin-2 is decreased in the collecting ducts of mice lacking the vasopressin V1a receptor. V1a receptor signaling may be fundamentally important for the expression of aquaporin-2 in the collecting ducts during control conditions and dehydration. However, in cases of kidney disease where existing diuretics such as conventional furosemide have proven ineffectual, tolvaptan may be effective, particularly in cases with hypoaalbuminemia. Tolvaptan may be effective for treating diabetic nephropathy based on the expression of aquaporin-2 in kidney tissue. Inasmuch as the small number of cases prohibits a definitive statement, these diseases may be indicated in the future for tolvaptan. Also, there are instances of intact medullary function even in cases with severe glomerular lesions in patients with advanced chronic kidney failure. Tolvaptan would also be indicated for such patients, based on the results showing that the collecting ducts are still functional. Tolvaptan therapy has been reported for massive edema in a patient with nephrotic syndrome. Tolvaptan increased urine and ultrafiltration volumes in patients with oliguria undergoing peritoneal dialysis. Therefore, tolvaptan may be a therapeutic option for the correlation of water balance in patients with diabetic nephropathy.

**Limitations:** The present study had several limitations. It was a retrospective study and the study population was small. Further investigations are needed to elucidate the relationship between the expression of aquaporin-2 and the efficacy of tolvaptan.

**Conclusion:** Tolvaptan is considered effective for some cases of diabetic nephropathy with heart failure. There are no clear parameters for predicting an effect, but the present study showed that aquaporin-2 was expressed in the epithelial cells of the collecting duct of tolvaptan responders.

**Acknowledgment**

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**References**

7. Inamura T, Kinugawa K, Shiga T, et al. Novel criteria of urine osmolality effectively predict response to tolvaptan in decompensated heart failure patients—association between non-responders and