Tolvaptan Prolongs Blockage of the Vasopressin Type II Receptor Over 24 Hours in Responders With Stage D Heart Failure

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SUMMARY

The urine aquaporin-2 (U-AQP2) level relative to the plasma arginine vasopressin (P-AVP) level is a novel predictor of the responsiveness to the vasopressin type 2 receptor (V2R) antagonist tolvaptan (TLV). However, little has been reported about the concentration-time profile of U-AQP2 after TLV treatment. We evaluated 24 patients with decompensated stage D heart failure (HF) who had received 3.75 mg/day of TLV on a *de novo* basis for > 7 days to treat congestion refractory to conventional diuretics. Seventeen patients were TLV-responders, whose 24-hour urine volume (UV) increased after TLV initiation; the other 7 patients were TLV-non-responders. The U-AQP2 of the TLV-responders, corrected for the urine creatinine concentration, decreased significantly at 4 hours after TLV administration without returning to the day-1 morning level on the morning of day-7. The TLV-non-responder U-AQP2 levels remained low even before the TLV treatment. On the morning of day-7, the TLV-responder U-AQP2/P-AVP ratio was comparable to that of the TLV-non-responders. Among 18 patients (11 responders and 7 non-responders), the day-7 TLV trough concentration was 64 ± 62 ng/mL and was negatively correlated with the estimated glomerular filtration rate (eGFR). TLV has antagonistic effects on the V2R over 24 hours in TLV-responders with advanced heart failure and chronic kidney disease, probably due to persistently elevated blood TLV concentration. The unresponsiveness to TLV in the TLV-non-responders is not attributable to malabsorption. (Int Heart J 2016; 57: 41-46)

Key words: Congestion, Drug responder, Renal failure

The arginine vasopressin (AVP) type 2 antagonist tolvaptan (TLV) is known to ameliorate congestion and to normalize hyponatremia, thereby preserving hemodynamics and renal function in patients with congestive heart failure (HF) refractory to a considerable number of conventional diuretics.¹⁻¹⁰⁾ In contrast, we sometimes experience TLV-non-responders in our daily clinical practice.¹¹⁾

We previously proposed a novel predictor of responsiveness to TLV, ie, the baseline urine aquaporin-2 (U-AQP2) level relative to the plasma AVP (P-AVP) level, which may indicate a preserved vasopressin type 2 receptor (V2R)-related signaling pathway in the principal cells of the renal collecting duct.^{12,13)} However, little is known about the concentration-time profile of U-AQP2 after the initiation of TLV treatment. Although Martin, *et al* observed the changes of U-AQP2 after the initiation of V2R antagonist, the responsiveness to TLV was not discussed.¹⁴⁾ Therefore, in this study, we investigated the U-AQP2 profile and the relationship between U-AQP2 and other clinical parameters after the initiation of TLV treatment in TLV-responders and TLV-non-responders.

METHODS

Patient selection: Among the patients who had been hospitalized at our institute between August 2013 and August 2014 for decompensated HF, data were obtained from 24 patients who had received 3.75 mg/day of TLV on a *de novo* basis for > 7 days. All patients presented New York Heart Association class III/IV symptoms accompanied by symptomatic congestion such as lower limb edema, pulmonary congestion, or jugular venous distention. The patients were refractory to guideline-directed medical treatment with a considerable number of conventional diuretics, including loop diuretics and/or thiazides in addition to the appropriate restriction of sodium and water intake. Therefore, all patients were classified as suffering from stage D heart failure.¹⁵⁾ Patients with any of the following conditions were excluded from the study and did not receive TLV: severe systemic infection or inflammation, severe stenotic valvular disease, end-stage renal failure or on hemodialysis, acute coronary syndrome occurring within a month, the need for the use of mechanical support, hypovolemia, impaired consciousness with lack of thirst, or hyponatremia (> 145 mEq/L of serum sodium) as previously described.¹¹⁾ The present study complies with the Declaration of Helsinki and the institutional review board of University of Tokyo approved the research

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protocol. Informed consent was obtained from all patients prior to enrollment.

Variables evaluated: Baseline demographic data were obtained within 24 hours prior to the TLV treatment. Blood samples, including those for the P-AVP measurement, were obtained in the early morning before the ingestion of medication (including diuretics) and after a 15-minute rest in the supine position.¹⁶⁾ Urine samples were collected at 3 time points: 1) in the early morning before the administration of any medication, including TLV and other diuretics (day-1 morning); 2) at 4 hours following the first TLV administration (day-1 4 hours); and 3) in the early morning at day-7 before the administration of any drugs, including TLV (day-7 morning). The U-AQP2 level was measured using a sandwich enzyme-linked immunosorbent assay (human aquaporin-2 ELISA kit, LSI Medience) and was corrected for the urine creatinine (U-Cre) concentration to allow for a quantitative comparison as previously described.^{12,17)} The urine volume (UV) collected during 24 hours after the initiation of the TLV treatment (day-1) was compared with that of the previous 24 hours (day-0). The patients, whose 24-hour UV at day-1 increased compared with that at day-0, were defined as TLV-responders (UV-defined responders). The other patients were assigned to the TLV-non-responders group (UV-defined non-responders).¹¹⁾ The TLV trough concentration

was measured in the blood samples obtained from 18 patients at day-7 in the morning using liquid chromatography-tandem mass spectrometry.¹⁸⁾

Statistical analyses: The data are expressed as the mean $\pm$ SD, unless otherwise indicated. The continuous variables were compared using unpaired Student's *t*-test or Mann-Whitney *U* test as appropriate. The categorical variables were compared using the χ^2 or Fisher's exact test as appropriate. Dunnett's test was used to compare the continuous variables among the several time points measured if repeated analysis of variance showed significance. Pearson's correlation coefficient was used to assess the correlation between 2 groups. All statistical analyses were performed using SPSS statistics software (version 22; SPSS, Inc., Chicago, IL, USA). All statistical tests were 2-tailed and $P < 0.05$ was considered significant.

RESULTS

Baseline characteristics: The baseline variables before the initiation of the TLV therapy are presented in Table I. The mean population age was 54 ± 20 years and the average estimated glomerular filtration ratio (eGFR) was 52 ± 15 mL/kg/1.73 m² (range: 23.8-69.4 mL/kg/1.73 m²). The TLV-responders had a

Table I. Baseline Variables Before the Initiation of TLV

	Total (<i>n</i> = 24)	Responders (<i>n</i> = 17)	Non-responders (<i>n</i> = 7)	<i>P</i>
Demographics				
Age, years	54 $\pm$ 20	51 $\pm$ 17	62 $\pm$ 24	0.202
Male, <i>n</i> (%)	19 (79)	13 (76)	6 (86)	0.612
BMI	22.9 $\pm$ 3.4	23.4 $\pm$ 3.2	22.8 $\pm$ 2.9	0.215
sBP, mmHg	96 $\pm$ 13	95 $\pm$ 13	100 $\pm$ 14	0.441
HR, bpm	82 $\pm$ 12	82 $\pm$ 12	83 $\pm$ 11	0.799
Medications				
BB, <i>n</i> (%)	21 (88)	15 (88)	6 (86)	0.865
ACEI, <i>n</i> (%)	16 (67)	11 (65)	5 (71)	0.751
AA, <i>n</i> (%)	15 (63)	12 (71)	3 (43)	0.202
Furosemide, <i>n</i> (%)	24 (100)	17 (100)	7 (100)	1.000
Furosemide, mg/day	65 $\pm$ 20	67 $\pm$ 21	59 $\pm$ 17	0.356
Laboratory				
Hemoglobin, g/dL	11.2 $\pm$ 2.1	11.6 $\pm$ 2.4	10.3 $\pm$ 0.9	0.087
Serum albumin, g/dL	3.4 $\pm$ 0.6	3.4 $\pm$ 0.5	3.3 $\pm$ 0.7	0.630
Serum sodium, mEq/L	134 $\pm$ 6	134 $\pm$ 7	135 $\pm$ 4	0.757
eGFR, mL/kg/1.73 m ²	52 $\pm$ 15	61.4 $\pm$ 45.1	35.5 $\pm$ 11.1	0.001*
Serum TB, mg/dL	1.3 $\pm$ 0.9	1.4 $\pm$ 0.9	1.3 $\pm$ 0.8	0.856
Plasma BNP, pg/mL	672 $\pm$ 648	711 $\pm$ 658	579 $\pm$ 663	0.659
Plasma AVP, ng/mL	3.9 $\pm$ 1.4	4.1 $\pm$ 1.3	3.5 $\pm$ 1.8	0.383
Urine variables				
U-OSM, mOsm/L	417 $\pm$ 122	464 $\pm$ 105	302 $\pm$ 79	0.001*
U-AQP2/U-Cre, $\times 10^2$	60 $\pm$ 42	76 $\pm$ 40	22 $\pm$ 9	< 0.001*
U-AQP2/P-AVP, $\times 10^3$	1.1 $\pm$ 0.8	1.5 $\pm$ 0.7	0.3 $\pm$ 0.1	< 0.001*
Echocardiography				
LVDd, mm	60 $\pm$ 14	61 $\pm$ 15	57 $\pm$ 15	0.317
LVEF, %	35 $\pm$ 18	34 $\pm$ 18	37 $\pm$ 19	0.234
NYHA class				
Class III, <i>n</i> (%)	11 (46)	7 (41)	4 (86)	0.476
Class IV, <i>n</i> (%)	13 (54)	10 (59)	3 (43)	0.476

BMI indicates body mass index; sBP, systolic blood pressure; HR, heart rate; BB, β -blocker; ACEI, angiotensin converting enzyme inhibitor; AA, aldosterone antagonist; eGFR, estimated glomerular filtration rate; TB, total bilirubin; BNP, B-type natriuretic peptide; AVP, arginine vasopressin; U-OSM, urine osmolality; U-AQP2, urine aquaporin-2; U-Cre, urine creatinine; LVDd, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association. * $P < 0.05$ by the unpaired *t*-test.

Figure 1. The concentration-time profiles of U-AQP2 (A), U-OSM (B), P-AVP (C), and U-AQP2/P-AVP (D) during TLV therapy in the TLV-responders and TLV-non-responders. R indicates TLV-responder; and NR, TLV-non-responder. * $P < 0.05$ compared with the baseline variable as determined by Dunnett' test if repeated analysis of variance showed significance. † $P < 0.05$ compared with the TLV-non-responders as determined by Tukey's test if 2-way repeated analysis of variance showed significance.

significantly higher urine osmolality (U-OSM) level, U-AQP2 level corrected for U-Cre, and U-AQP2/P-AVP ratio than those of the TLV-non-responders ($P < 0.05$ for all comparisons).

Time-dependency of U-AQP2 levels and of other variables: In the TLV-responders, both the U-AQP2 level (Figure 1A) and the U-OSM level (Figure 1B) markedly decreased at 4 hours following the first administration of TLV ($P < 0.05$ for both analyses). The U-AQP2 and U-OSM levels at day-7 before the administration of TLV were significantly lower as compared with the day-1 morning levels ($P < 0.05$ for both analyses). In contrast, in the TLV-non-responders, the U-AQP2 and U-OSM levels remained low and were not affected by the TLV treatment at all 3 time points measured. The P-AVP level was increased at 4 hours after the first administration of TLV and remained at a higher level at day-7 in the TLV-responders (Figure 1C). As a result, the TLV-responder U-AQP2/P-AVP ratio was similar to that of the TLV-non-responders at day-7 morning (Figure 1D; 0.6 ± 0.4 versus $0.4 \pm 0.3 \times 10^3$, $P = 0.087$).

TLV trough concentration at day-7: The TLV trough concentration was measured in 18 patients, including 11 responders and 7 non-responders, at day-7 morning (64 ± 62 ng/mL on average). No significant differences were observed in the TLV trough concentration between the TLV-responders and TLV-non-responders (43 ± 38 versus 98 ± 80 ng/mL, $P = 0.182$). As shown in Table II, only the eGFR was significantly correlated with the TLV trough levels at day-7 morning among both responders and non-responders to TLV (Figure 2; $P = 0.019$, $r =$

Table II. Relationship Between Baseline Variables and Trough Level of TLV at 1 Week Following TLV Administration

	<i>P</i>	<i>r</i>
Demographics		
Age, years	0.211	-0.312
BMI	0.132	0.371
sBP, mmHg	0.763	-0.076
HR, bpm	0.215	-0.307
Medications		
Furosemide, mg/day	0.143	-0.365
Laboratory		
Hemoglobin, g/dL	0.525	-0.162
Serum albumin, g/dL	0.2021	0.316
Serum sodium, mEq/L	0.734	-0.086
eGFR, mL/kg/1.73 m ²	0.019*	-0.545
Serum total bilirubin, mg/dL	0.658	0.112
Plasma BNP, pg/mL	0.611	-0.129
Plasma AVP, ng/mL	0.434	0.243
Echocardiography		
LVDd, mm	0.243	0.143
LVEF, %	0.265	0.176

Abbreviations as in Table I. * $P < 0.05$ by Pearson's correlation coefficient.

-0.545). The TLV trough level was negatively correlated with U-AQP2 in the responders ($P = 0.042$, $r = -0.342$), but not in the non-responders ($P = 0.946$, $r = 0.065$).

Figure 2. The relationship between the eGFR and the TLV trough concentration at day-7. White and red circles indicate the TLV-responders and TLV-non-responders, respectively. * $P < 0.05$ as determined by Pearson's correlation coefficient.

DISCUSSION

This study showed that the U-AQP2 level decreased 4 hours after the administration of TLV in the TLV-responders, whereas it remained low in the TLV-non-responders. Furthermore, the responder U-AQP2 level at day-7 did not fully return to the baseline level. The TLV trough concentration at day-7 was measured in 18 patients: that of the TLV-non-responders was as high as that of the TLV-responders.

Excretion of AQP2 in urine: The precise function of AQP2 has been investigated in several early, classical analysis studies. AQP2 is the AVP-regulated water channel that mediates water transport across the apical plasma membrane of the renal collecting duct.¹⁹ The number of AQP-2 water channels determines the water permeability of the apical membrane and is principally regulated by the P-AVP level.²⁰ AVP regulates the translocation of AQP-2 proteins from cytosolic vesicles to the apical plasma membranes, a shuttle trafficking process that regresses with the suppression of AVP stimulation.²¹ Approximately 3% of AQP2 in the kidney is excreted in the urine daily; this fraction is not affected by the various conditions present in patients.²² Therefore, U-AQP2 is considered a marker of collecting duct responsiveness to AVP.^{12,23}

U-AQP2 and responsiveness to TLV: We previously demonstrated that responsiveness to TLV is largely dependent on the viability of the collecting duct,¹¹ and that TLV-responders can be discriminated from TLV-non-responders by measuring their respective baseline U-AQP2 relative to P-AVP level prior to TLV administration.¹² However, few reports exist on the concentration-time profile of U-AQP2 after the initiation of TLV therapy. Martin, *et al* previously reported that the U-AQP2 level decreased significantly after the administration of TLV,¹⁴ however, the potential relationship between the changes in the U-AQP2 level and the responsiveness to TLV remains unknown.

Acute U-AQP2 response: In the TLV-responders, we observed decreases in the U-AQP level at 4 hours following the administration of TLV. The 4-hour time point was chosen based on previous studies showing that both the maximum plasma concentration of TLV and the maximum UV response occurred at 4 hours.²⁴ AVP stimulates the synthesis of AQP2 and a part of

this is excreted in urine. A 4-hour observation time is enough to see the changes in transcription of AQP2. We confirmed that the inhibition of the V2R by TLV resulted in a marked decrease in the U-AQP2 level, suggesting a reduced activation, ie, less phosphorylation and translocation, of AQP2. Importantly, the altered concentration of AQP2 in the urine was not merely a result of aquaresis but rather a consequence of a real decrease in solute excretion, since we corrected the U-AQP2 concentration for that of urine creatinine. In contrast, an already low U-AQP2 level was observed in the TLV-non-responders before the TLV treatment, which was not decreased after TLV administration. This finding suggests that V2R-mediated signaling pathways are not active in the TLV-non-responders, or at least do not propagate through the principal cells of the renal collecting duct. We speculate that the unresponsiveness to TLV is attributable to a certain dysfunction of the collecting duct, which is often observed in aged patients with decompensated HF.¹¹

Mid-term U-AQP2 response: The P-AVP level increased at day-7. Although the precise mechanism underlying the increase in P-AVP after the administration of TLV remains unknown, such an increase is consistently observed.⁸ Although TLV therapy elevates serum osmolality by increasing serum sodium levels, it is usually insufficient to stimulate the excretion of AVP.¹⁶ P-AVP can also be secreted by non-osmotic stimulation, including hypotension, but TLV rarely induces hemodynamic compromise even in patients with advanced HF.⁵

Despite the increase in the P-AVP level at day-7, the U-AQP2 level did not return to the baseline level after 7 days of TLV administration (75% compared with that at baseline) in the TLV-responders, indicating that the AQP2 excretion in urine was considerably suppressed after 24 hours of TLV administration. Since the antagonistic effect of TLV is transient (personal communication from Otsuka Pharmaceuticals), we hypothesize that TLV at its measured trough concentration at day-7 can inhibit the V2R in the presence of AVP. Here, we measured the TLV trough concentration at day-7 because the plasma TLV concentration is known to remain stable for at least 7 days.¹⁸

At day-7 morning, the average TLV trough concentration in 18 patients, including 11 responders and 7 non-responders, was 64 ng/mL, which was higher than those of healthy subjects or HF patients with normal kidney function.^{8,25} Van Wart, *et al* observed a more elevated peak plasma concentration of TLV in HF patients with or without liver dysfunction compared with that of healthy subjects.²⁶ Moreover, Kida, *et al* reported an elevated TLV trough level at day-7 in HF patients with chronic kidney disease.¹⁸ Most of the patients in the current study had chronic kidney disease in addition to stage D HF. Although the precise mechanism underlying the persistently elevated TLV trough concentration in such conditions is still unknown, a decreased systemic circulation and over volume due to renal impairment may contribute to the reduced metabolism of TLV and elevated blood TLV concentration. The TLV trough level in the TLV-non-responders was similar to that of the TLV-responders. This result supports our hypothesis that the unresponsiveness to TLV was not attributable to the low blood concentration of TLV due to malabsorption, but rather to the dysfunction of the renal collecting duct.^{11,12} Accordingly, the up-titration of TLV may not be effective in the TLV-non-responders.

We previously proposed the criterion to predict responsiveness to TLV: the U-AQP2 level relative to the P-AVP level, which can be obtained before the initiation of TLV therapy.¹²⁾ As shown in the present study, the U-AQP2/P-AVP ratio at day-7 was similarly decreased and low in the TLV-responders and the TLV-non-responders. It is therefore important that these biomarkers should be measured before the initiation of TLV treatment.

Study limitations: 1) The present study was performed in a small population in a single study center. Hence, the findings should be confirmed in a future multi-center, large-scale population study. 2) The present study was conducted at a fixed TLV dose. Dose comparison analyses may provide additional information. However, the adjustment of each different TLV dose to the patients' background may be difficult in a real clinical setting. 3) We analyzed the mid-term U-AQP2 response at 7 days after the initiation of the TLV treatment, assuming that the TLV trough concentration had reached steady state at that time point. Determining the U-AQP2 levels after long-term V2R inhibition by TLV (eg, over years) is the next step to be taken. 4) We enrolled patients with New York Heart Association class III or IV and who had comorbid reduced renal function. Therefore, our results cannot be extrapolated to relatively less sick patients with a normal eGFR. 5) Martin, *et al* observed the changes of U-AQP2 every 2 hours until 8 hours after V2R antagonist administration,¹⁴⁾ whereas we measured it at 4 hours and day-7. AVP can stimulate synthesis of AQP2, and 4 hours may be enough to observe increased urinary secretion of AQP2 by way of AVP-enhanced transcription of AQP2. The continuous measurement of UV using a urethral catheter may provide information regarding the precise responsiveness to TLV, although the use of a dwelling urethral catheter has become uncommon. 6) U-OSM data are obtained more rapidly than U-AQP2. U-OSM may have an advantage in the rapid prediction of responsiveness to TLV. However, U-AQP2 relative to P-AVP had better predictability compared with U-OSM or U-OSM/P-AVP (area under curve for predictability, 1.000, 0.871, and 0.639, respectively). 7) Baseline U-AQP2/P-AVP was obtained in the responders receiving anti-HF treatment including diuretics ($n = 17$, $1.5 \pm 0.7 \times 10^3$), and the data represent chronic effects of baseline anti-HF treatment on U-AQP2/P-AVP. We also obtained a comparable level of U-AQP2/P-AVP in a healthy population receiving no anti-HF treatment ($n = 5$, $2.4 \pm 1.4 \times 10^3$, $P = 0.635$). Therefore, general anti-HF treatments do not seem to affect the level of U-AQP2/P-AVP, which may mostly reflect the ability of the collecting duct. However, there are no data about the changes of U-AQP2/P-AVP after the administration of anti-HF treatment such as diuretics. U-AQP2/P-AVP was affected not only immediately but also chronically by the administration of TLV as shown in this study. On the other hand, U-AQP2/P-AVP did not appear to be affected by the chronic anti-HF treatment including conventional diuretics when U-AQP2/P-AVP was measured in the early morning before taking any medicine. However, we do not know if U-AQP2/P-AVP is changed immediately after taking such anti-HF agents. Therefore, U-AQP2/P-AVP should be measured in the early morning before taking any medications including conventional diuretics as well as TLV. 8) We did not measure several urine parameters including beta-2 microglobulin and N-acetyl-beta-D-glucosaminidase, which are known as indices of tubular dysfunction. Such data would clarify the

specific relationship between the collecting duct function and responsiveness to TLV.

Conclusion: TLV can persistently antagonize the V2R and may provide aquaresis over 24 hours in TLV-responders with chronic kidney disease, probably due to the impaired renal excretion of the drug.

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DISCLOSURE

K.K. has a potential conflict of interest with Otsuka Pharmaceutical Co., which consists of lecture fees and endowments from the Department K.K. works at.

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