

Efficacy of tolvaptan on nephrotic patients with diuretic-resistant edema: a pilot study

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Research article

Keywords: nephrotic syndrome, vasopressin receptor type 2 antagonist, aquaporin 2, diuretics, furosemide-resistant, urine output

Posted Date: September 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-52612/v1>

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Abstract

Background Tolvaptan (TLV), a vasopressin type 2 receptor antagonist, is an effective drug for heart failure without worsening renal function. However, the effect of TLV on nephrotic syndrome remains unknown. This study aims to assess the association between response to TLV and clinical parameters including pathological evaluation in nephrotic patients with diuretics-resistance.

Methods For prospectively enrolled patients, TLV was added for 14 days after renal biopsy performed. We evaluated the effect of TLV on urine output (UO) and body weight (BW) and the correlation of UO change with BW, urine and blood measurements and pathological evaluation. Pathological evaluation included glomerular sclerosis, tubulointerstitial injury, interstitial fibrosis and the degree of aquaporin 2 (AQP2) positivity in collecting ducts.

Results Ten nephrotic patients were enrolled. Two patients already received 140 and 160mg furosemide and others 40mg furosemide before TLV. After TLV administration, no patients showed worsening renal function or hypernatremia (>145 mEq/L) and two patients without any improvement of symptoms discontinued the trial. TLV significantly increased UO at day 1 and decreased BW overall. UO increase at day 1 was significantly correlated with BW decrease at the last follow-up. UO increase at day 1 was correlated only with AQP2 positivity in collecting ducts on biopsied kidney ($r^2=0.59$, $p=0.01$) while other urine and blood measurements were not.

Conclusions TLV was effective on nephrotic patients. UO increase by TLV was correlated with AQP2 positivity in the collecting ducts while biochemistry in urine and blood was not.

Background

Tolvaptan (TLV) is an antagonist of vasopressin type 2 receptor and promotes water diuresis. The first study reports that TLV is effective for the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) to correct hyponatremia¹. And now TLV plays an important role in treating patients with acute and chronic heart failure refractory to other diuretics². Responders to TLV experienced not only a better clinical course but better prognosis in both short- and long- term observation³. Moreover, TLV has no adverse effect on neurohormonal activation or renal activation while other diuretics can exaggerate neurohormonal imbalance and cause vasoconstriction with the reduction of renal perfusion. The previous clinical trial showed TLV helped weight loss and better relief of dyspnea without worsening renal function in heart failure patients⁴.

Nephrotic syndrome is also a state of fluid overload with decreased urine volume and often accompanied with renal dysfunction. Although the pathophysiology of sodium and water retention in nephrotic syndrome has not been precisely clarified, activation of the renin-angiotensin aldosterone system and sympathetic nervous system is referred as a part of causes. Underfilled circulation to kidneys activates these neurohormonal systems and may worsen renal function⁵. Vasopressin type 2 receptor antagonists,

which are not expected to cause neurohormonal imbalance, will be an ideal agent to treat hypervolemia in nephrotic syndrome without worsening renal function⁶.

Although some case reports showed TLV was effective in patients with nephrotic syndrome^{7,8}, it has not been fully elucidated which nephrotic patients are responsive to TLV. Also, nephrotic patients sometimes have renal impairment^{9,10}, which is one of the major risk factors for resistance to TLV¹¹. Thus, it is required to evaluate whether nephrotic syndrome is responsive to TLV and whether the response to TLV can be predicted by urine, blood or pathological test.

This study aims to evaluate 1) responsiveness for TLV treatment and 2) associating factors including serum and urinary biochemical parameters, urinary aquaporin (AQP) 2, serum TLV concentration, renal pathological findings, and renal AQP2 expression for TLV effectiveness, in patients with diuretics-resistant nephrotic syndrome.

Methods

Patient enrollment

The inclusion criteria were (a) nephrotic syndrome and (b) fluid overload resistant to diuretics. Nephrotic syndrome was defined as > 3.5 g/day proteinuria and hypoalbuminemia (< 3.0 g/dL). Diuretic resistance was defined that volume overload remained even when furosemide dose was 40 mg or more. The exclusion criteria were patients with allergy to vaptans, anuria (< 200 mL/day), difficulty to free water intake, hypernatremia (> 147 mEq/L), or pregnancy.

Study Protocol

This was a prospective exploratory study conducted from January through December in 2014. The study design is shown as Supplemental Figure S1. Evaluation of the degree of edema, chest X-ray, echocardiogram, and renal biopsy was performed at the screening phase before TLV administration. Then TLV 7.5 mg per day was added on enrolled patients for 14 days. During the trial, other diuretics than TLV were continued with unchanged doses. If urine output did not increase or symptoms were not improved for two days after 7.5 mg of TLV administration, the dose of TLV could be increased to 15 mg as needed. During TLV administration, water intake was not restricted to prevent volume depletion.

Body weight, daily urine output, blood pressure and pulse rate were checked every day. Serum and urine osmolality, serum sodium and creatinine were measured before TLV administration, and at 4hr, 8hr, day2, 3, 5, 8, and 15 after TLV medication.

Patients were followed up until day 15 if TLV was effective and safe. Follow up was finished at the time when symptoms were not improved or exaggerated and continuing trial was considered as a risk during the trial.

This study protocol was approved by Tokushukai Group Institutional Review Board (TGE00343-024) and adhered to the Declaration of Helsinki and to CONSORT guidelines. Informed consents were obtained before the study registration by written form. This study was also registered in UMIN-CTR (UMIN000011763).

Measurement of urine AQP2, serum TLV concentration and anti-diuretic hormone (ADH).

Urine AQP2 before TLV administration was determined using sandwich enzyme-linked immunosorbent assay (human AQP2 ELISA kit, LSI Medience) as described previously^{12,13}. Serum TLV concentration was evaluated at 4 hour after TLV administration on day 1, and determined using a validated high-performance liquid chromatography-tandem mass spectrometry method at Toray Research Center, Inc. Details have been described previously¹⁴. Plasma ADH before TLV administration was determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits

Pathological evaluation

To evaluate the collagen deposition, 2 µm sections of paraffin-embedded tissue were subjected to Masson Trichrome staining (MTS) by routine procedures. Stained sections were examined by Olympus BX50 epifluorescence microscope equipped with a digital camera DP73 (Tokyo, Japan). To estimate fibrotic area, computer-aided morphometric analysis on MTS sections was performed as described previously¹⁵. Briefly, a grid containing 117 (13 × 9) sampling points was superimposed on images of a cortical high-power field (x400). The number of grid points overlying the MTS-positive area was counted and expressed as a percentage of all sampling points. For each kidney specimen, 10 randomly selected non-overlapping fields were analyzed.

To evaluate glomerular sclerosis, 2 µm sections of paraffin-embedded tissue were subjected to hematoxylin-eosin staining by routine procedures. All glomeruli in tissue slice were evaluated at × 400 using a semiquantitative scoring method as follows: grade 0, no obvious sclerosis (normal); grade 1, sclerotic area 25% (minimal sclerosis); grade 2, sclerotic area 25–50% (moderate sclerosis); grade 3, sclerotic area 50–75% (moderate-severe sclerosis); and grade 4, sclerotic area 75–100% (severe sclerosis). The glomerulosclerotic index (GSI) was calculated using the weighted average of > 10 glomeruli¹⁶.

Tubulointerstitial injury in the cortex was analyzed histomorphometrically by counting the number of tubules that demonstrated vacuolar degeneration, chromatin condensation of tubular nuclei, tubular dilatation and thickened and/or wrinkled tubular basement membranes divided by the number of total tubules per field (× 200) in 10 randomly selected cortical fields per cross Sect.¹⁷.

To compare the histological features in participants, three patients with minor glomerular abnormality and normal renal function were evaluated as controls (N = 3). The pathological evaluation was performed by well-trained nephrologists.

Immunohistochemical staining and evaluation of AQP2 immunostaining

Two micrometer of formalin-fixed specimen were used for AQP2 immunostaining. After deparaffinization, endogenous peroxidase activity was blocked by 0.3% H₂O₂ in methanol for 20 minutes at room temperature. Then sections were incubated with Rabbit anti-human AQP2 (C-17) antibody (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) for primary antibody for an hour at room temperature. Bound antibodies were detected with peroxidase-conjugated goat anti-rabbit antibody (Histofine Simple Stain MAX PO; Nichirei Biosciences, Tokyo, Japan) using diaminobenzidine-tetrahydrochloride (DAB) (Simple Stain DAB solution; Nichirei Biosciences, Tokyo, Japan) as the substrate for 30 minutes at room temperature. The sections were counterstained with hematoxylin. The normal part of the whole kidney with clear cell carcinoma was used for normal control.

We defined the proportion of AQP2 staining as the proportion of the number of stained cells divided by the number of all cells in a sectional collecting duct. The example in Supplemental Figure S2 shows five AQP2-positive cells (red arrows) of twelve cells so that the proportion of AQP2 staining is 0.42. AQP2 positivity was evaluated in all collecting ducts in each kidney specimen in each patient.

Statistical analysis

Data were presented as median (interquartile). Wilcoxon ranked test was used for rejecting the null hypothesis that the change of UO and BW was not significant. Wilcoxon ranked test was used when comparing between time-serial data on osmolality and electrolytes in urine and serum because all data were not normally distributed. The existence of correlation between two continuous variables was determined using Spearman correlation. We did statistical analyses by JMP Pro 11 for Windows (SAS Institute Japan, Tokyo). A significant difference was defined as p-value < 0.05.

Results

Baseline characteristics

Ten patients with nephrotic syndrome were enrolled and Table 1 describes their baseline characteristics. Two patients already received 140 and 160 mg furosemide and others 40 mg furosemide before TLV. Diabetic nephropathy was the majority in the causes of nephrotic syndrome and 9 patients had renal impairment. No one had very low cardiac output (EF < 30%). The median of urine osmolality was lower than the predictive value for responsiveness to TLV in the previous report ¹⁸.

Table 1
Baseline characteristics

| Age (y) | 66 (49–70) |
|---|---------------------|
| M/F | 7/3 |
| Height (cm) | 166.5 (151.8–174.3) |
| Weight (kg) | 79.4 (70.8–90.2) |
| Causes | |
| DN | 8 (80%) |
| MCNS | 2 (20%) |
| Hypertension | 6 (60%) |
| NYHA classification | |
| II | 3 (30%) |
| III | 4 (40%) |
| IV | 3 (30%) |
| Nohria classification | |
| B | 9 (90%) |
| C | 1 (10%) |
| Systolic blood pressure (mmHg) | 133 (123–171) |
| Heart rate (/min) | 68 (64–87) |
| Ejection Fraction (%) | 60 (41–67) |
| Diameter of IVC at exhalation (mm) | 4.7 (4.1–11.9) |
| Diameter of IVC at inhalation (mm) | 18 (15.2–21.7) |
| Total protein (g/dL) | 5 (4.1–5.5) |
| Albumin (g/dL) | 2.0 (1.8–2.2) |
| UN (mg/dL) | 21.2 (18.9–32.2) |
| Creatinine (mg/dL) | 1.79 (1.32–2.43) |
| eGFR (mL/min/1.73 m ²) | 27.5 (21.2–43.1) |
| Sodium (mEq/L) | 140 (138–142) |
| ADH, anti-diuretic hormone; AQP2, aquaporin 2; BNP, brain natriuretic peptide; DN, diabetic nephropathy; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; IVC, inferior vena cava; MCNS, minimal change nephrotic syndrome; TLV, tolvaptan; UN, urea nitrogen | |

| Age (y) | 66 (49–70) |
|---|-------------------|
| Potassium (mEq/L) | 3.9 (3.4–4.2) |
| Chloride (mEq/L) | 103 (102–108) |
| Total Cholesterol (mg/dL) | 181 (153–232) |
| Triglyceride (mg/dL) | 90 (74–131) |
| LDL-C (mg/dL) | 119 (75–185) |
| HDL-C (mg/dL) | 43.7 (39.3–49.0) |
| Hemoglobin A1c (%) | 6.3 (5.8–8.3) |
| Serum osmolality (mOsm/kg) | 289 (285–293) |
| BNP (pg/mL) | 321 (74–663) |
| ADH (pg/mL) | 2.1 (1.6–4.0) |
| Renin (ng/mL/hr) | 0.6 (0.4–2.0) |
| Aldosterone (pg/mL) | 43.8 (33.1–63.6) |
| Urine osmolality (mOsm/kg) | 321 (258–337) |
| Urinary protein (g/day) | 6.86 (4.16–9.46) |
| Urine UN (mg/dL) | 327 (139–507) |
| Urine creatinine (mg/dL) | 57.4 (35–117) |
| Urine sodium (mEq/L) | 68.5 (48.5–94.5) |
| Urine potassium (mEq/L) | 15.6 (11.8–28.1) |
| Urine chloride (mEq/L) | 56.5 (43–109) |
| Urine AQP2 (ng/mL) | 0.83 (0.57–1.31) |
| TLV concentration (ng/mL) | 95.2 (64.9–134.3) |
| ADH, anti-diuretic hormone; AQP2, aquaporin 2; BNP, brain natriuretic peptide; DN, diabetic nephropathy; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; IVC, inferior vena cava; MCNS, minimal change nephrotic syndrome; TLV, tolvaptan; UN, urea nitrogen | |

Five patients finished follow-up before day 15 because of no improvement of symptoms (N = 2) or discharge in alive (N = 3). The remaining five patients were followed up until day 15.

The pathological evaluation was exhibited in Supplemental Figure S3. When compared with patients with minor glomerular abnormality and normal renal function, nephrotic patients had more glomerular

sclerosis and tubulointerstitial injury, while interstitial fibrosis was not significantly different between 2 groups.

Osmolality and electrolytes in urine and serum after TLV administration

The time-dependent course of osmolality and electrolytes in spot urine, and serum are depicted in Supplemental Figure S4. Spot urine osmolality and electrolytes significantly decreased four and eight hours after TLV administration and returned to baseline at day 2 or after. On the other hand, except the increase of serum osmolality at day 2 and serum chloride at day 2–3, serum osmolality and electrolytes were unchanged during TLV administration. All patients did not suffer from any electrolyte disorders including hypernatremia (> 145 mEq/L) and did not have worsening renal function with repeatitive TLV administration.

Effect and safety of TLV in patients with nephrotic syndrome

Urine output at day 1 exhibited a significant increase from baseline and urine output after day 1 was not significantly different from baseline. Body weight was significantly reduced at the last follow-up after TLV administration (Fig. 1).

When the correlation with urine output and weight change at last follow-up was evaluated, the change of urine output at day 1 was significantly correlated with weight change at last follow-up ($R^2 = 0.52$, $p = 0.02$).

No association with urine output increase by TLV and various measurements in urine and blood.

Although some measurements in urine and blood changed after TLV administration (Supplemental Figure S4), the change of UO at day 1 was not correlated with osmolality and electrolytes in urine and serum before TLV administration (Table 2). Also, the change of urine output on day 1 did not correlate with urine AQP2 before administration. As same as urine AQP2, the concentration of TLV and plasma ADH were not related to the change of urine output. (Table 3)

Table 2:

The correlation of urine output change at day 1 with urine and serological measurements.

None of them were associated with urine output.

| | R ² | p-value |
|------------|----------------|---------|
| Spot urine | | |
| Osmolality | 0.03 | 0.62 |
| Sodium | 0.04 | 0.58 |
| Potassium | 0.01 | 0.74 |
| Chloride | 0.07 | 0.48 |
| Protein | 0.004 | 0.86 |
| Creatinine | 0.002 | 0.90 |
| UN | 0.01 | 0.75 |
| Serum | | |
| Osmolality | 0.05 | 0.52 |
| Creatinine | 0.07 | 0.45 |
| UN | 0.04 | 0.56 |
| Urine AQP2 | 0.07 | 0.45 |
| Plasma ADH | 0.05 | 0.58 |
| Serum TLV | 0.03 | 0.65 |

Abbreviations: AQP2, aquaporin 2; ADH, antidiuretic hormone; TLV, tolvaptan; UN, urea nitrogen.

Table 3
The correlation of urine output change on day 1 with pathological evaluation.

| | R² | p-value |
|---|----------------------|----------------|
| Spot urine | | |
| Osmolality | 0.03 | 0.62 |
| Sodium | 0.04 | 0.58 |
| Potassium | 0.01 | 0.74 |
| Chloride | 0.07 | 0.48 |
| Protein | 0.004 | 0.86 |
| Creatinine | 0.002 | 0.90 |
| UN | 0.01 | 0.75 |
| Serum | | |
| Osmolality | 0.05 | 0.52 |
| Creatinine | 0.07 | 0.45 |
| UN | 0.04 | 0.56 |
| Urine AQP2 | 0.07 | 0.45 |
| Plasma ADH | 0.05 | 0.58 |
| Serum TLV | 0.03 | 0.65 |
| Pathological parameters | R² | p-value |
| Fibrosis | 0.01 | 0.79 |
| GSI | 0.11 | 0.36 |
| TI | 0.08 | 0.42 |
| AQP2 | 0.59 | 0.01 |
| Abbreviations: GSI, glomerular sclerosis index; TI, tubulointerstitial injury | | |

Pathological evaluation revealed the degree of AQP2 staining was related to urine output

Then urine output change was evaluated from the pathological point. The proportion of AQP2 staining was strongly correlated with urine output change at day 1 while the degree of fibrosis, glomerular

sclerosis and tubulointerstitial injury were not significantly correlated with urine output change at day 1 (Table 3).

Discussion

Nephrotic syndrome is common kidney disease. Some nephrotic patients have refractory edema resistant to near-maximum doses of diuretics^{19,20}. Also, volume correction and the use of diuretics may deteriorate renal function^{21,22}. Optimal treatment for nephrotic edema without worsening renal function is an important issue. An antagonist to V2 receptor, TLV, blocks water reabsorption via aquaporin 2 in cortical collecting ducts without activating the renin-angiotensin-aldosterone axis and the sympathetic nervous system⁶. TLV is expected as a novel drug for nephrotic edema. However, to date, it remains to be clarified who is responsive to TLV though some researchers reported the effect of TLV on nephrotic edema. Then our study challenged to elucidate the association of responsiveness to TLV with pathological evaluation and serological or urinary markers. And the novel findings are as below. First, urine output change at day 1 was correlated with the degree of weight loss at the final. Second, the proportion of AQP2 staining was correlated with the increase of urine output by TLV while urine and blood measurements including uAQP2 and TLV were not correlated. And finally, TLV could be used safely for some nephrotic patients without adverse effects in the short term.

Previous reports showed that responders to TLV in heart failure patients had a significant increase in urine volume soon after administration and improved clinical course with significant weight loss²³. As similar to the previous studies, our study demonstrated that weight loss was also significantly related to the increase of urine volume at day 1 in nephrotic syndrome. This indicated that urine volume soon after TLV administration was enough to evaluate whether it was responsive to TLV and the clinical course was improved in nephrotic syndrome patients.

A transient increase in urine output on day 1 was not reflected in a concomitant weight loss on day 2. This undesirable result is attributed to arbitrary water intake. In general, fluid restriction is needed for volume control irrespective of the use of diuretics. However, at the time when this study was conducted, Pharmaceuticals and Medical Devices Agency in Japan did not recommend fluid restriction to avoid volume depletion. Moreover, we could not predict the urine output increase so that we could not manage volume control appropriately. But the result in this study revealed the effect of TLV and we would manage the proper fluid restriction.

Urine AQP2, urine osmolality change and serum TLV concentration can be predictors for TLV efficacy in heart failure^{18,24,25}. However, these measurements did not correlate with urine output change and weight loss in our study despite detected urine AQP2, the reduction of urine osmolality and elevated TLV concentration. Moreover, serum ADH was not correlated with the effect of TLV. There were no precise explanations, but some reasons could be speculated. First, evidence was not enough about the association with urine AQP2 and pathological AQP2 staining in nephrotic syndrome though AQP2 expression in kidney tissues and net urine AQP2 were increased in a previous report²⁶. Urine output in

nephrotic patients sometimes decreases and urine AQP2 concentration needs to be adjusted when it is interpreted. However, AQP2 is located and excreted in principal cells of collecting ducts. Urine creatinine or other filtration makers were not enough as calibrations because they do not reflect the dynamics of collecting ducts precisely. Thus, urine AQP2 concentration did not correlate with AQP2 staining and urine output change in nephrotic patients. Second, the majority in our study had renal impairment, unlike previous reports. Patients with decreased eGFR disable to dilute or concentrate urine despite elevated vasopressin concentration²⁷, which indicates collecting ducts in the diseased kidney are reluctant to respond to vasopressin and V2 receptor antagonists. Serum ADH is slightly higher in CKD patients, which indicates that the response to ADH would be sluggish. Although the mechanism of urine dilution and concentration is not clearly understood, it is considered that urine osmolality, serum ADH or TLV concentration were not enough to predict the aquauresis by TLV.

Although the finding in this study indicates that it requires a renal biopsy to predict urine output change by TLV in nephrotic patients, renal biopsy is impractical only for the purpose. Biopsy has several risks such as flank pain, infection and bleeding. A few days are necessary for the result of the biopsy. Thus, the finding in this study may makes little clinical sense and a novel, easier manner will be expected for predicting the responsiveness. However, although it may not give a suggestion to our clinical practice immediately, we believe that this finding in this study is academically important. Our findings are not totally consistent with previous studies because we found that the effect of tolvaptan retained for patients with kidney dysfunction, but was correlated with AQP2 in biopsied kidney, not urine and blood parameters like the previous studies. Thus, we consider this finding should be described in this article to further understand the physiology of diuresis under kidney impairment.

The limitation of this study is a small exploratory study in a single center for the short term. The population in this study was limited to nephrotic patients with diabetic nephropathy and CKD stage 3–4. The pathophysiology of diabetic nephropathy and nephrotic patients may be different as indicated in the previous report²⁸. It is postulated that nephrotic syndrome results from permeability factors affecting podocyte function such as cardiotrophin-like cytokine 1, which is not regarded as a cause of diabetic nephropathy^{29,30}. Therefore, this study cannot tell that tolvaptan is effective for idiopathic nephrotic syndrome and further studied are required. Second, the number of enrolled patients could not be reached to the target size (N = 20) during the trial. More detail evaluation for the larger population and other types of nephrotic syndrome is needed. The long term effect of TLV should be evaluated in further study. Third, this study was an exploratory study, not a randomized trial with controls of conventional therapy. Further randomized comparative trials are needed to validate the efficacy of tolvaptan. Fourth, low dose TLV in this study may be a potential limitation. According to the report on the pharmacokinetics of TLV, even higher dose TLV have less effect on urine excretion in advanced kidney disease³¹. Therefore, higher dose might have increased urine output in some patients without any improvement of symptom in this study.

Conclusion

Our exploratory prospective study provided that TLV increased urine output in nephrotic patients and the increase of urine output by TLV was only correlated with AQP2 positivity in kidney tissue. Urinary and serological measurements were not correlated with the effect of TLV, and no clinical parameters except kidney biopsy specimen may predict the responsiveness for TLV in patients with nephrotic syndrome.

Declarations

Ethics approval and consent to participate:

This study protocol was approved by Tokushukai Group Institutional Review Board (TGE00343-024) and adhered to the Declaration of Helsinki. Informed consents were obtained before the study registration by written form. Trial registration: UMIN-CTR, UMIN000011763. Registered 20 August 2013, <https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&recptno=R000013754&type=summary&language=J>

Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

Dr. Koichiro Kinugawa received honorarium from Otsuka Pharmaceutical co. Other authors declared no competing interests

Funding. None

Author's contribution

RM and TO conceived and designed the study, analyzed the data, and drafted the manuscript. YM, KI, KM, and MO conducted the data collection. SH, TI, KK and SK provided additional guidance for the analysis. All authors revised the manuscript critically for important intellectual content and gave final approval of the manuscript.

Acknowledgment. None.

Consent for publication: Not Applicable

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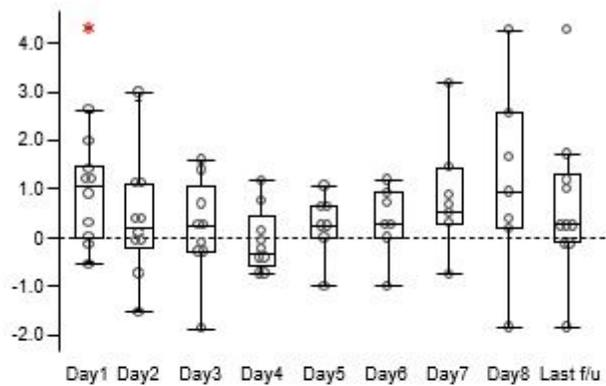
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Figures

(a) The change of UO compared to baseline (L/day)



(b) The change of BW compared to baseline (kg)

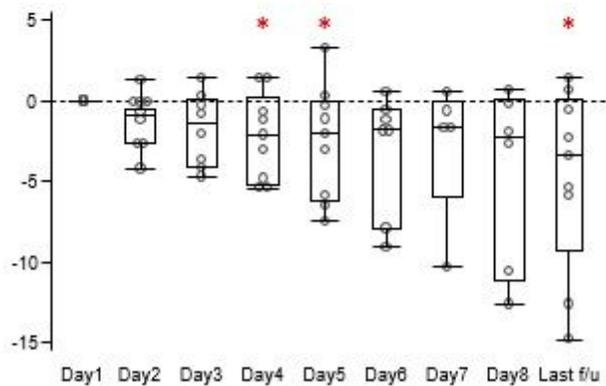


Figure 1

The change of urine output and body weight in a day after TLV administration. This figure shows the time-dependent course of the change of (a) urine output (mL/day) and (b) body weight (kg) compared to those before TLV administration. (a) The significant increase of urine output was observed only at day 1. (b) The significant decrease in body weight was observed at day 4, 5 and last follow-up. Box plot shows median and interquartile range. * $p < 0.05$.

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