Strategies to optimize renoprotective therapy in proteinuric renal patients

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Chapter 8

Effect of the urotensin receptor antagonist palosuran on albuminuria in hypertensive patients with type 2 diabetic nephropathy: results from the PROLONG proof-of-concept study

**Background** — The urotensin system has been hypothesized to play an important role in the pathophysiology of diabetic nephropathy. PROLONG, a multicenter, randomized, double-blind, placebo-controlled, 2-period, crossover study, assessed the effects of the urotensin receptor antagonist palosuran on urinary albumin excretion (UAE) and blood pressure in hypertensive patients with type 2 diabetic nephropathy treated either with an angiotensin II receptor type 1 (AT1) antagonist or an angiotensin-converting enzyme (ACE) inhibitor.

**Methods** — Patients with macroalbuminuria (24-h UAE > 0.5 and < 3.0 g) and systemic hypertension (supine systolic blood pressure ≥ 135 and < 170 mmHg and/or diastolic ≥ 85 and < 110 mmHg) received both palosuran 125 mg twice daily and placebo for 4 weeks.

**Results** — Out of the 62 enrolled patients, 54 were included in the per-protocol population: male/female: 43/11, mean age: 61.6 years, (geometric) mean UAE: 1016 mg/24h, mean systolic/diastolic blood pressure: 155/84 mmHg, mean creatinine clearance: 84 mL/min. Palosuran did not affect UAE: the palosuran ratio (UAE at end of treatment over UAE at baseline) over placebo ratio (geometric mean) was 0.99 (95% CI: 0.85, 1.14). Similarly, palosuran did not affect placebo-subtracted systolic or diastolic casual blood pressure: -1.9 mmHg (16.5) and -0.2 mmHg (9.5), respectively. Renal function remained stable throughout the study.

**Conclusion** — In hypertensive patients with type-2 diabetic nephropathy, treated either with an AT1 antagonist or an ACE inhibitor, combined treatment with the urotensin receptor antagonist palosuran did not affect albuminuria or blood pressure. These results suggest that inhibition of the urotensin system may not represent a new treatment strategy in this high-risk patient population.

Type 2 diabetic patients with overt nephropathy and systemic hypertension are characterized by elevated cardiovascular and renal risk. The widely accepted standard treatment strategy in these patients includes at least one inhibitor of the renin-angiotensin-aldosterone system (RAAS), an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin II type 1 receptor (AT1) antagonist, as both classes of drugs have been proven to reduce cardiac and renal morbidity and mortality. In patients treated with a RAAS inhibitor, the risk reduction in morbidity and mortality is correlated with the extent of blood pressure and albuminuria decrease (1,2). However, the residual cardiovascular and renal risk in patients treated with an ACE inhibitor (3,4) or an AT1 antagonist (5,6) remains high. Despite attempts to improve RAAS blockade by combining ACE inhibitor and AT1 antagonist treatment, patients still progress to end-stage renal disease (7). Therefore, still an unmet medical need for new treatments to be combined with a RAAS inhibitor in this high-risk patient population is present.

The recently discovered urotensin system has been hypothesized to play an
important role in the pathophysiology of cardiac and renal diseases (8). In the kidney, human urotensin II and the urotensin receptor are mostly expressed in glomeruli and capillary endothelial cells (9,10). Urotensin II, initially described as the most potent vasoconstrictor known in mammals (8,11), exerts its effects by modulating vascular tone (12-14), but these effects vary according to vascular bed. In the kidney, one *in vivo* rat study reported that infusion of urotensin II reduced renal plasma flow (RPF) and GFR (15,16), while in another study intra-renal infusion of urotensin II led to an increase in RPF (17). In patients with diabetic nephropathy, both urotensin II and urotensin receptor gene expression were up-regulated in kidney tissue (18), and renal dysfunction was associated with elevated plasma urotensin II levels (9,19,20).

Palosuran (ACT-058362) is an oral, selective, competitive, non-peptidic antagonist of the human urotensin receptor. Palosuran was studied both in animal and humans to assess the role of the urotensin system in the pathogenesis of diabetic nephropathy. In different rat models of renal failure, palosuran has been shown to prevent the development of renal failure by improving RPF and renal vascular resistance, GFR, and serum creatinine, and by reducing glomerular and tubulo-interstitial lesions, without affecting systemic blood pressure (21,22). In a diabetic rat model, palosuran delayed development of albuminuria and improved survival (22).

In humans, data from phase I studies in healthy adult males showed that palosuran was well tolerated and had no effects on systemic blood pressure, heart rate, or plasma urotensin II levels (23,24). Apart from an open-label pilot study in patients with type 2 diabetic nephropathy suggesting that palosuran might reduce albuminuria (25), the effects of palosuran in patients were largely unknown. Based on these preliminary preclinical and clinical data, it was hypothesized that the urotensin system might be a mediator in the pathogenesis of diabetic nephropathy, and that its inhibition would have a protective effect on the kidney. Therefore, PROLONG, a multicenter, randomized, double-blind, placebo-controlled, 2-period crossover, proof-of-concept study was designed to assess whether palosuran would reduce urinary albumin excretion (UAE) and/or systemic blood pressure in type hypertensive patients with type 2 diabetic nephropathy on stable treatment with either an ACE inhibitor or AT1 antagonist.

**METHODS**

**Patients**

Patients of both sex, between 30 and 75 years of age, with type 2 diabetes (with or without insulin treatment) and Hb A1c < 10%, systemic hypertension (supine systolic blood pressure ≥ 135 and < 170 mmHg and/or diastolic ≥ 85 and < 110 mmHg), macroalbuminuria (24-h UAE ≥ 0.5 and < 3.0 g), and a measured creatinine clearance ≥ 30 ml/min/1.73 m², were randomized to this 2-period crossover study.
Patients had to be on stable treatment with either an ACE inhibitor or AT1 antagonist for at least 3 months. Any treatment with diuretics, calcium channel blockers, beta-blockers, statins and non-steroidal antiinflammatory drugs, had to be stable for at least one month before screening. Finally, patients should have had no history of clinically relevant worsening of renal function in the last 6 months. Patients could not be included if they were women of childbearing potential, were treated with a combination of an AT1 antagonist and an ACE inhibitor, had clinically relevant signs of: nephrotic syndrome; significant renal artery stenosis; moderate to severe hepatic dysfunction; serum albumin < 25 g/L; serum potassium $\geq$ 5.5 mmol/L; or a urinary tract infection within one month before screening. Ethics committees approved the study according to national regulations. All patients gave their written informed consent.

**Study design**
PROLONG was a randomized, multicenter, double-blind, placebo-controlled, 2-period crossover study. After screening, eligible patients entered a 4-week run-in period. Thereafter, patients were randomly treated during two treatment periods of 4 weeks with palosuran (ACT-058362) 125 mg and placebo twice daily separated by a 6-week washout period. Finally, patients entered a 4-week follow-up recovery period. At the end of each study period, patients were instructed not to take their trial medication in the morning of that day. A permuted block method was used for randomization.

**Determination of UAE, renal function and blood pressure**
To reduce the known high intra-individual variability of UAE, UAE was assessed as the mean albuminuria calculated from 3 consecutive 24-h urine collections measured locally using the same center-specific measurement method throughout the study. Creatinine clearance was calculated from serum creatinine and mean 24-h urinary creatinine excretion. Trough supine systemic blood pressure was measured every 3 min during 15 min by an automated oscillometric device. Blood pressure assessment was the mean of the three last measurements.

In addition, a monocenter ancillary study has been performed in the 22 patients included at the Mario Negri institute in Ranica, Italy. Renal plasma flow and glomerular filtration rate were measured by inulin and para-aminohippurate clearance methods, respectively. Blood pressure was measured by 24-h ambulatory blood pressure monitoring (ABPM).

**Statistics analysis**
As PROLONG was designed as a proof-of-concept study, the main analysis was performed on the per-protocol population, which included all randomized patients who
Table 1. Demographic and baseline characteristics at screening

<table>
<thead>
<tr>
<th>Per protocol population</th>
<th>( n = 54 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male / Female (n)</td>
<td>43 (80%) / 11 (20%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.6 (8.4)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>91.8 (16.9)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>31.7 (5.3)</td>
</tr>
<tr>
<td>Systolic/diastolic blood pressure (mmHg)</td>
<td>155.2 (15.8) / 84.5 (8.6)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.49 (1.45)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>84.4 (36.4)</td>
</tr>
<tr>
<td>UAE (mg/24hour)</td>
<td>1016 (58)</td>
</tr>
<tr>
<td>Duration of type 2 diabetes (years)</td>
<td>16.2 (9.5)</td>
</tr>
<tr>
<td>Duration of hypertension (years)</td>
<td>14.4 (10.7)</td>
</tr>
<tr>
<td>Duration of macroalbuminuria (years)</td>
<td>4.0 (4.6)</td>
</tr>
<tr>
<td>Use of ACE inhibitor (n)</td>
<td>36 (67%)</td>
</tr>
<tr>
<td>Use of AT1 antagonist (n)</td>
<td>18 (33%)</td>
</tr>
</tbody>
</table>

* Data are expressed as arithmetic mean with standard deviation (SD), except UAE expressed as geometric mean with coefficient of variation (CV%).

completed both treatment periods and had complete UAE data. Based on previous clinical experience, a 30% (log-transformed: 0.357) difference in the mean change from baseline in the 24-h UAE was considered clinically relevant. Standard deviation (SD) was estimated to be 1 on the logarithmic scale. A total of 50 patients were needed to demonstrate this hypothesis, using a two-sided paired t-test with type-1 error of 10% and a power of 80%. Data are expressed as arithmetic means with standard deviation, except for the UAE and UAE over creatinine ratio, which were log-transformed prior to analysis to take into account the asymmetric distribution of UAE. The UAE and UAE over creatinine ratio are expressed as geometric mean with coefficient of variation (CV%).

RESULTS

A total of 62 patients was included in the PROLONG study conducted between February 2004 and April 2005 in 11 centers from Italy (\( n = 24 \)), The Netherlands (\( n = 10 \)), Israel (\( n = 12 \)), Australia (\( n = 7 \)), France (\( n = 7 \)), and Switzerland (\( n = 2 \)). Of the 62 patients, 54 completed the study according to the protocol, 28 were randomized first to placebo followed by palosuran, whereas 26 received first palosuran followed by placebo. The reasons for excluding 8 patients from the per-protocol analysis were: no valid or missing UAE data (\( n = 4 \)), low compliance to the treatment (\( n = 2 \)) and no treatment received during the second treatment period (\( n = 2 \)). Demographic and baseline characteristics at screening are summarized in table 1.
Efficacy

Results are summarized in table 2. No period effect (i.e., the disease remains stable), or treatment carry over effect were observed in this crossover study. The mean UAE remained stable during the total study duration (figure 1). The intra and inter-patient variability of UAE were high but not different to those observed in other studies (30). Compared to placebo, palosuran did not significantly affect UAE: end of treatment over baseline ratio of 1.0 (95% CI: 0.93, 1.15) and 1.02 (95% CI: 0.93, 1.12), respectively, with a palosuran ratio over placebo ratio (geometric mean of log-transformed data) of 0.99 (95% CI: 0.85, 1.14). The use of the urinary albumin over creatinine ratio to correct for potential urine collection bias did not modify the results. Similarly, palosuran did not affect placebo-subtracted systolic or diastolic blood pressure: -1.9 mmHg (16.5) and -0.2 mmHg (9.5), respectively, or heart rate. In the ancillary study, data from 19 patients out of 22 confirmed the absence of clinically relevant effect on blood pressure measured by 24-hour ambulatory blood pressure monitoring. Palosuran had no effect on serum glucose levels.

Renal function measured by serum creatinine and creatinine clearance, and urinary electrolytes (sodium and potassium) remained stable after palosuran or placebo treatment. In the ancillary study, per-protocol data from 20 patients out of 22, did not show any significant difference between palosuran and placebo on RPF and GFR, measured by inulin and para-aminohippurate clearance methods, respectively. With palosuran RPF decreased from 325.0 to 298.2 ml/min/1.73m² and GFR decreased from

### Table 2. Results

<table>
<thead>
<tr>
<th>Per protocol population n = 54</th>
<th>Placebo</th>
<th>Palosuran</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>154.2 (17.0)</td>
<td>155.0 (18.0)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83.3 (10.4)</td>
<td>84.3 (10.4)</td>
</tr>
<tr>
<td>UAE * (mg/24h)</td>
<td>1047 (76)</td>
<td>1084 (77)</td>
</tr>
<tr>
<td>UAE/creatinine * (mg/mmol)</td>
<td>87 (78)</td>
<td>90 (85)</td>
</tr>
<tr>
<td>Albumin fractional clearance</td>
<td>37 (31)</td>
<td>40 (37)</td>
</tr>
<tr>
<td>Serum creatinine (umol/L)</td>
<td>122 (43)</td>
<td>120 (41)</td>
</tr>
<tr>
<td>Serum urea (mmol/L)</td>
<td>9.8 (4.9)</td>
<td>9.6 (4.6)</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>4.3 (0.5)</td>
<td>4.3 (0.5)</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>79 (30)</td>
<td>80 (33)</td>
</tr>
<tr>
<td>Urinary urea (mmol/24h)</td>
<td>653 (339)</td>
<td>661 (360)</td>
</tr>
<tr>
<td>Urinary sodium (mmol/24h)</td>
<td>190 (67)</td>
<td>181 (62)</td>
</tr>
<tr>
<td>IR-hUII (pg/mL)</td>
<td>1.5 (1.3)</td>
<td>1.4 (1.3)</td>
</tr>
</tbody>
</table>

BP, blood pressure. UAE, urinary albumin excretion. IR-hUII, immunoreactive human urotensin II. * Data are expressed as arithmetic mean with standard deviation (SD), except UAE and UAE over creatinine ratio expressed as geometric mean with coefficient of variation (CV%).
69.6 to 68.6 ml/min/1.73m², while with placebo, RPF decreased from 326.5 to 304.6 ml/min/1.73m² and GFR decreased from 66.9 to 62.2 ml/min/1.73m².

Plasma urotensin II levels measured at the end of palosuran treatment did not show any identifiable pattern as compared to baseline.

Safety
In the 62 patients who received the study drug, the number of patients with at least one adverse event was 14 (23%) and 17 (27%) when treated with placebo and palosuran, respectively. The most frequently reported adverse events were headache (4.8 vs. 1.6%), nausea (3.2 vs. 3.2%), and first-degree atrioventricular block (3.2 vs. 1.6%) during palosuran and placebo treatment, respectively. During the study, no adverse event led to study drug discontinuation and no patient died. Two serious adverse events not considered by the investigators to be related to study drug were reported: one basal cell carcinoma and one cerebrovascular accident.

Discussion
PROLONG was the first placebo-controlled study assessing the effect of the urotensin receptor antagonist palosuran in diabetic nephropathy. Although previous preclinical and clinical studies suggested that blockade of the urotensin system might be beneficial in hypertensive patients with type 2 diabetic nephropathy, palosuran did not show any effect on UAE or casual blood and 24-h ambulatory blood pressure, in patients treated with either an ACE inhibitor or AT1 antagonist. Renal function, measured as creatinine clearance and serum creatinine as well as RPF and GFR in the ancillary study, remained stable.
These neutral results do not support the hypothesis that an antagonist of the urotensin receptor such as palosuran would counteract the deleterious effects of urotensin II in the pathophysiology of diabetic nephropathy. However, these findings may provide additional insight into the putative role of the urotensin system in the cardio-renal physiology and pathophysiology, still subject to debate (12-14).

Similarly to angiotensin II, human urotensin II was thought to contribute to kidney damage, by successively triggering hyperfiltration, albuminuria, glomerular and interstitial lesions. Indeed, it has been shown that the urotensin system was upregulated in patients with diabetic nephropathy (18), or various degrees of renal dysfunction (9,19,20). However, preclinical and clinical studies with intravascular infusion of urotensin II showed different results. In rats, intravenous administration of urotensin II reduced renal blood flow and GFR (15,16), but intra-renal infusion lead to an increase in renal blood flow, abolished by nitric oxide synthase inhibition (17). In humans, intravenous infusion of urotensin II in healthy volunteers had no effect on forearm blood flow in one study (26), whereas another study showed a dose-dependent decrease in forearm blood flow (27). These conflicting data suggest that urotensin II may influence vascular tone by different mechanisms, i.e. direct vasoconstriction and/or indirect vasodilatation via the release of nitric oxide or prostacyclin (17,29).

Preclinical studies with different urotensin receptor antagonists showed more consistent results suggesting that the urotensin system may be an important mediator in the development of nephropathy (16,21,22). In different rat models of diabetic and non-diabetic nephropathy, it has been shown that palosuran prevented the development of renal failure, improved RPF and GFR, delayed development of albuminuria and reduced glomerular and tubulo-interstitial lesions (21,22). In addition, a pilot study in hypertensive patients with type-2 diabetic nephropathy treated either with an ACE inhibitor or AT1 antagonist, showed that palosuran administered during 2 weeks decreased UAE by 24%, without affecting RPF and GFR, or systemic blood pressure (25). Of note, the assessment of UAE was not the primary objective of this open-label, non-comparative study, its assessment in this study (one 24-h urine collection) may explain the discrepant results compared to the PROLONG study.

Finally, the study results should be interpreted knowing the limited available data on the role of the urotensin system in the pathophysiology of nephropathy. Firstly, since all patients were on stable treatment with an inhibitor of the RAAS, the independent effects of urotensin receptor blockade could not be studied. In addition, potential interactions between the two hormonal systems have not been investigated. Therefore, one cannot rule out that the urotensin system plays a minor role when the RAAS is inhibited. Secondly, no dose-titration for albuminuria or blood pressure was performed in this study. Thus, a too low dose of palosuran might have been given in this study.
Thirdly, while all proven antiproteinuric agents exert their effects within several weeks (29), one cannot rule out that the time of exposure was too short to observe an effect on albuminuria. However, at that time, the available preclinical toxicology data did not allow a longer exposure of patients to palosuran.

In summary, these results suggest that inhibition of the urotensin system does not seem to represent a new treatment strategy in hypertensive patients with type 2 diabetic nephropathy. The inhibition of the RAAS by an ACE inhibitor, an AT1 antagonist or their combination, remains the cornerstone in treatment modalities protecting cardiac and renal function in this high-risk patient population.

CONTRIBUTORS

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CONFLICTS OF INTEREST

Actelion Pharmaceuticals Ltd has sponsored the PROLONG study. The investigators had the opportunity to interpret the data and write the related publication independently from Actelion.

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