TRV120027, a Novel Beta-Arrestin Biased Ligand at the Angiotensin II Type I Receptor, Unloads the Heart and Maintains Renal Function When Added to Furosemide in Experimental Heart Failure

Boerrigter et al: TRV120027 and Furosemide in Heart Failure

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Abstract

**Background**—TRV120027 is a novel β-arrestin biased ligand of the angiotensin II type 1 receptor (AT1R); it antagonizes canonical G protein-mediated coupling while, in contrast to classical AT1R antagonists, it engages β-arrestin-mediated signaling. Consequently, TRV120027 inhibits angiotensin II-mediated vasoconstriction while, via β-arrestin coupling, it increases cardiomyocyte contractility. We hypothesized that TRV120027 would elicit beneficial cardiorenal actions when added to furosemide in experimental heart failure (HF).

**Methods and Results**—Two groups of anesthetized dogs (n=6 each) with tachypacing-induced HF were studied. After a baseline clearance, one group (F+V) received furosemide (1 mg/kg/h) plus saline for 90', while the other (F+T) received the same dose of furosemide plus TRV120027 (0.3 and 1.5 μg/kg/min for 45' each); two clearances were done during drug infusion. After a washout, a post-infusion clearance was done. *p<0.05 between groups. F+V and F+T increased diuresis and natriuresis to a similar extent during the drug administration, but urine flow* and urinary sodium excretion* were higher in the post-infusion clearance with F+T. Glomerular filtration rate was preserved in both groups. Renal blood flow increased with F+T but this was not significant vs. F+V. Compared to F+V, F+T decreased mean arterial pressure*, systemic* and pulmonary* vascular resistances, and ANP*. Pulmonary capillary wedge pressure* decreased to a larger extent with F+T than with F+V.

**Conclusions**—When added to furosemide, TRV120027, a novel β-arrestin biased AT1R ligand, preserved furosemide-mediated natriuresis and diuresis, while reducing cardiac preload and afterload. These results provide support for TRV120027 as a promising novel therapeutic for the treatment of HF.

**Key Words:** animal models of human disease, heart failure, cardiovascular pharmacology, angiotensin II, receptors, beta-arrestin
Heart failure (HF) is a common disease with increasing prevalence which continues to be associated with high morbidity and mortality. Volume overload in acute decompensated HF (ADHF) is usually treated with loop diuretics, which, while frequently efficacious in mobilizing fluids, can be associated with diuretic resistance and worsening renal function. Renal dysfunction and worsening renal function are powerful independent predictors of worse outcomes in HF. While diuretic resistance and worsening renal function may simply identify sicker patients, it is also possible that our current therapeutic strategies are suboptimal or even harmful. Given the continuing substantial mortality and rehospitalization rates for ADHF, new, innovative strategies that enhance or protect cardiac and renal function in ADHF are an urgent need.

Angiotensin II plays an important role in the pathogenesis of HF and inhibition of its actions by angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) have shown beneficial effects in the treatment of patients with chronic HF. Angiotensin II mediates many of its actions via the angiotensin II type I receptor (AT1R), which belongs to the family of 7 transmembrane helical receptors which are also referred to as G protein coupled receptors. Recent studies have shown that the AT1R signals not only via G proteins, but also via β-arrestins. Importantly, it has been recognized that AT1R ligands can be biased toward β-arrestin signaling with unique functional consequences, which holds exciting promise to develop drugs that more precisely target the specific needs of patients. For example, an unbiased AT1R blocker such as losartan will antagonize the vasoconstricting effects of angiotensin II, but it will also block distinct, potentially beneficial pharmacology mediated by β-arrestin signaling. In contrast, the β-arrestin biased AT1R ligand TRV120027, a peptide with a short half-life
currently in clinical development for ADHF, antagonizes G protein coupling but engages β-arrestin signaling. In rodents, this results in vasodilation and increased cardiac contractility while decreasing myocardial oxygen consumption.27

We have recently reported the cardiorenal actions of TRV120027 in normal and HF canines.28 In the canine HF model, which recapitulates most clinical features of HF, TRV120027 effects were consistent with in vitro and rodent pharmacology, leading to decreased mean arterial pressure, decreased pulmonary capillary wedge pressure, and increasing cardiac output. Since loop diuretics are commonly used to manage volume status in patients with ADHF, we sought to understand the potential interplay of furosemide and TRV120027 on hemodynamic and renal parameters in a canine HF model. We hypothesized that TRV120027 would have beneficial hemodynamic actions while preserving renal function in canines with experimental HF treated with furosemide.

Methods

Studies were performed in two groups of male mongrel dogs (weight 20.5–30 kg; n=6 per group) in accordance with the Animal Welfare Act and with the approval of Mayo’s Institutional Animal Care and Use Committee. Animals were maintained on a sodium controlled diet (Hill’s I/d diet, Hill’s pet nutrition, Topeka, KS).

Animals had a pacemaker implanted with an epicardial lead on the right ventricle as previously described in detail.29 After at least 10 days of recovery, pacemakers were programmed to stimulate at 240 beats per minute. An acute experiment was done on day 11 of pacing. On the evening before the acute experiment, dogs were fasted and given 300 mg of lithium carbonate orally for later assessment of renal tubular function. On the day of the
experiment, the pacing was discontinued and animals were anesthetized with pentobarbital and fentanyl, endotracheally intubated, and mechanically ventilated (rate 12/min, tidal volume 15 mL/kg body weight) with supplemental oxygen. Lines were inserted into the femoral vein for infusion of inulin, saline, and study drugs. A line was advanced via the femoral artery to measure arterial pressure and for blood sampling. A balloon-tipped, flow-directed thermodilution catheter was advanced into the pulmonary artery via the jugular vein to measure cardiac filling pressures and cardiac output. Via a left flank incision and retroperitoneal dissection a catheter was inserted into the ureter for timed urine collections. After surgical preparation, pacing at 240 beats per minute was reintroduced. The renal artery was equipped with an electromagnetic flow probe to measure renal blood flow. A weight adjusted inulin bolus was administered and a continuous inulin infusion (1 mL/min) and a saline infusion (1 mL/min) were started. After 60 minutes of equilibration, a 30-minute baseline clearance (C1) was done. All clearances consisted of urine collection, hemodynamic measurements, and blood sampling midway through the clearance. After the baseline clearance, the saline infusion was stopped and an infusion with furosemide (1 mg/kg/h, infusion rate 0.5 mL/min) was started. In addition, animals were randomly assigned to one of two groups. One group, F+V, received an infusion of vehicle (saline at 0.5 mL/min). In contrast, the other group, F+T, received TRV120027 (Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala-OH; 0.3 μg/kg/min, diluted in saline, infusion rate 0.5 mL/min). After a 15-minute lead-in, a second 30-minute clearance (C2) was done. After this the F+V group continued to receive saline (0.5 mL/min), while the F+T group received TRV120027 at 1.5 μg/kg/min (diluted in saline, infusion rate at 0.5 mL/min). Again, after a 15-minute lead-in, a 30-minute clearance (C3) was done. After that, furosemide and vehicle or TRV120027 infusions were stopped and replaced with a saline infusion (1 mL/min). After a 30-minute
washout period, a final 30-minute post-infusion clearance (C4) was performed. Pressures and renal blood flow were recorded digitally and analyzed offline (Sonometrics Corporation, London, ON, Canada). Cardiac output was measured by thermodilution (Cardiac output model 9510-A computer, American Edwards Laboratories, Irvine, CA). Renal blood flow was measured by electromagnetic flow probe (Carolina Medical Electronics, King, NC). Renal perfusion pressure was calculated as mean arterial pressure – right atrial pressure.

**Assays**

Electrolytes were measured by flame photometry (IL943, Instrumentation Laboratories, Lexington, MA). Inulin was measured with the anthrone method. ANP, BNP, angiotensin II, plasma renin activity, and aldosterone were measured as previously described. Proximal fractional reabsorption of sodium was calculated with the lithium clearance technique as follows: 
\[
[1 - (\text{lithium clearance} / \text{GFR})] \times 100
\]
Distal fractional reabsorption of sodium was calculated as: 
\[
[(\text{lithium clearance} - \text{sodium clearance}) / \text{lithium clearance}] \times 100.
\]

**Statistical analysis**

Values provided are mean ± standard error of the mean or median (25th/75th percentile). Changes within group were analyzed with one way analysis of variance for repeated measurements with post-hoc Dunnett’s test; alternatively, Friedman’s test with post-hoc Dunn’s test was used for not normally distributed data. Differences between groups were analyzed by comparing the changes from baseline with unpaired t-test or, if data were not normally distributed, with Mann-Whitney U-test. Statistical significance was accepted at p<0.05.
Statistical analyses were done with GraphPad Prism software (Prism 5.02 for Windows, GraphPad, San Diego, CA).

Results

Results are provided in the Table and Figures 1 and 2. At baseline, both groups showed a HF phenotype with decreased cardiac output, increased systemic vascular resistance, sodium retention, and neurohumoral activation.\textsuperscript{30}

Hemodynamic Function

In the F+V group mean arterial pressure, cardiac output, and systemic vascular resistance did not significantly change compared to the respective baseline values, but mean arterial pressure tended (p=0.07) to decrease in C4 (Fig. 1A-C). In contrast, in the F+T group mean arterial pressure decreased in C2 and C3 compared to baseline (p<0.05 vs. F+V) and returned to levels seen with F+V in the post-infusion clearance (Fig. 1A). Cardiac output tended (p=0.07) to increase with F+T (Fig. 1B; not significant vs. F+V), while systemic vascular resistance decreased during C2 and C3 (Fig. 1C; p<0.05 vs. F+V). Right atrial pressure decreased in both experimental groups with no difference between groups. Pulmonary artery pressure also decreased in both groups, but more so with F+T in C2 and C3. Pulmonary vascular resistance was unchanged with F+V but decreased with F+T, which was significant between groups for C2 and C3 (Fig. 1D). Pulmonary capillary wedge pressure decreased in both groups but more so with F+T during C3 (Fig. 1E). Left ventricular external work was unchanged with F+V but tended to decrease during infusion of TRV120027 (p=0.16; not significant between groups).
Renal blood flow and renal vascular resistance remained unchanged with F+V, while renal blood flow increased and renal vascular resistance decreased vs. baseline with F+T; however, these changes were not significant compared to F+V. Renal perfusion pressure was unchanged with F+V but decreased with F+T during C2 and C3, and this was significant between groups. Of note, all significant hemodynamic changes induced by TRV120027 returned to values not significantly different from F+V in the post-infusion clearance C4

**Renal Function**

In the F+V group, urine flow and urinary sodium excretion increased during C2 and C3 (Fig. 2A and B, respectively). With F+T, urine flow and urinary sodium excretion increased to similar degrees during C2 and C3 but were still significantly increased compared to baseline and compared to F+V in C4. A similar pattern was seen for urinary potassium excretion. Proximal and distal fractional sodium reabsorption (Fig. 2C) were decreased during C2 and C3, and, to a lesser extent in C4 with F+V. The same pattern was seen with F+T; however, distal fractional sodium reabsorption was significantly lower with F+T in C4 as compared to F+V. Glomerular filtration rate tended to increase (p=0.10) with F+V and was maintained with F+T despite the reduction in renal perfusion pressure.

**Neurohumoral Function**

Hematocrit increased compared to baseline in the F+V group and remained unchanged with F+T with no significant differences between groups. Plasma renin activity decreased with F+V in the post-infusion clearance, while it remained unchanged with F+T; however, plasma renin activity was significantly higher with F+T vs F+V in C3. F+V tended to increase
aldosterone during C3 (p=0.11), while it remained unchanged with F+T; however aldosterone
tended to be higher with F+V vs. F+T during C3 (p=0.065; Fig. 2D). Angiotensin II levels
tended to increase in the F+V group compared to baseline (p=0.004, post-hoc test not
significant); angiotensin II could not be measured reliably for the F+T group due to cross-
reactivity of TRV120027 with the assay. ANP and BNP remained unchanged with F+V while
they tended to decrease with F+T (ANP: p=0.09; BNP: p=0.11); compared to F+V, F+T
significantly reduced ANP in C3 (Fig. 2E).

Discussion

We report for the first time the cardiorenal actions of TRV120027, a novel β-arrestin
biased AT1R ligand, when added to the loop diuretic furosemide in experimental HF. Compared
to furosemide alone, addition of TRV120027 to furosemide unloaded the heart without adversely
affecting GFR, renal excretory function, or increasing aldosterone. Indeed, with TRV120027 the
increases in urine flow and in urinary sodium excretion were more sustained, atrial natriuretic
peptide decreased, and aldosterone tended to decrease.

Loop diuretics have been used for decades to treat sodium retention and volume overload
in patients with ADHF. While they are able to induce a potent diuresis, diuretic therapy in
ADHF is frequently associated with worsening renal function, which portends a poorer
prognosis. Indeed, higher diuretic doses have been associated with worse outcomes in
retrospective studies; however, diuretic dosage may simply be a marker of a more severely
diseased patient population. In the recent prospective DOSE trial, a high-dose strategy as
compared to a low-dose strategy of loop diuretic administration was associated with a trend for
better symptom improvement, a higher incidence of worsening renal function but no difference
in 60-day outcomes (death, rehospitalization, or emergency room visit).\textsuperscript{2} Importantly, mortality and hospital readmission rates were disconcertingly high in all study groups, emphasizing that novel therapeutic strategies to treat patients with ADHF are urgently needed.

As mentioned above, TRV120027 is a novel drug with unique properties at the AT\textsubscript{1R}.\textsuperscript{27} Similar to conventional angiotensin II type I receptor blockers (ARBs) it blocks G protein coupling but in contrast to ARBs it engages $\beta$-arrestin signaling. Unlike conventional ARBs, TRV120027 is not an orally available small-molecule drug, but it is a peptide, with consequently short half-life and the need for parenteral administration. Like angiotensin II, TRV120027 is an octapeptide and in fact the two peptides are identical in 6 amino acids. However, one important difference between them is the amino acid at the amino-terminus (phenylalanine in angiotensin II, D-alanine in TRV120027). Most recently, this amino acid has been found to be crucial for differentially engaging G-protein signaling, $\beta$-arrestin signaling, and stabilizing the complex between $\beta$-arrestin and the AT\textsubscript{1R} in endosomes.\textsuperscript{26} Dosing rats with TRV120027 resulted in dose-dependent vasodilation, increased cardiac contractility, and decreased myocardial oxygen consumption.\textsuperscript{27} We recently reported that TRV120027 in canines with experimental HF decreased cardiac preload and afterload, systemic and renal vascular resistances, and left ventricular external work while increasing cardiac output and renal blood flow. GFR and renal excretory function were maintained.\textsuperscript{28} Importantly, the reduction in mean arterial pressure was rapidly reversible, consistent with the approximate 2-minute half-life of TRV120027.\textsuperscript{28} These properties make TRV120027 an attractive potential drug for ADHF, in which cardiac unloading, enhancement of cardiac function, and preservation of renal function are high priorities. In addition, the ability to rapidly control the pharmacologic actions of the drug is also critical to avoiding potentially deleterious prolonged hypotension.\textsuperscript{31-33} In the current study, we sought to
test TRV120027 in a clinically relevant setting and therefore we added TRV120027 to
furosemide in experimental HF.

In this study, consistent with earlier studies, TRV120027 acted as an arterial vasodilator,
as evidenced by the dose-dependent reduction in mean arterial pressure and systemic vascular
resistance. This vasodilatory effect was rapidly reversible. In the post-infusion clearance mean
arterial pressure in both groups were similar, suggesting that the reduction of mean arterial
pressure in the post-infusion clearance compared to baseline is a time effect or secondary to the
furosemide-induced diuresis, not a persistent effect of TRV120027. The decrease in right atrial
pressure was not statistically significant which indicates that TRV120027’s vasodilatory actions
on the arterial vasculature are more prominent than its effects on the venous system. The
observed reduction in pulmonary capillary wedge pressure could be due to the afterload
reduction or venodilation. While not significant compared to the furosemide+vehicle group,
cardiac output tended to increase compared to baseline in the furosemide+TRV120027 group.
This may be secondary to improved cardiac preload and afterload but, as in vivo and in vitro data
have demonstrated, a positive effect on cardiac contractility via β-arrestin signaling may also
play a role.27 Pulmonary vascular resistance was also decreased with addition of TRV120027,
which may be of benefit to the many ADHF patients who have concomitant pulmonary arterial
hypertension and right heart failure.34 Taken together, these hemodynamic data suggest that
TRV120027 may help to unload the left and right heart. This is also supported by the observed
decrease in ANP: ANP is secreted by the heart in response to cardiac stress and thus a decrease
can be interpreted as a neurohumoral indicator of cardiac unloading. BNP measures in this study
were more variable than ANP measures, but BNP also tended to decline in the F+T group
compared to the F+V group.
TRV120027 increased renal blood flow compared to baseline, but this was not significant compared to the furosemide alone group. Renal vascular resistance tended to be lower compared to furosemide alone during C2. Despite the significant reduction in renal perfusion pressure, urine flow, and urinary sodium and potassium excretion were maintained throughout C2-C4 compared to furosemide alone. GFR tended to increase in the furosemide alone group, which could be due to its inhibition of tubular glomerular feedback (TGF), which under physiological conditions decreases GFR when sodium delivery is increased to the juxtaglomerular apparatus. Of note, increased sodium delivery to the distal nephron with long term administration of loop diuretics can lead to hypertrophy and hyperplasia of distal nephron segments, which are insensitive to the actions of furosemide; however, the clinical importance of these changes is not well known. Addition of TRV120027 maintained GFR, despite the reduction in renal perfusion pressure, which would generally be expected to decrease hydrostatic filtration pressure in the glomerulus. However, the hydrostatic pressure promoting filtration in the glomerulus not only depends on the renal perfusion pressure, but also on the differential tone of the afferent and efferent glomerular arteriole. Interestingly, angiotensin II in most situations reduces renal blood flow but maintains GFR by vasoconstricting the efferent arteriole, while having little effect on the afferent arteriole, thus increasing glomerular filtration pressure. How a β-arrestin biased AT1R ligand like TRV120027 affects afferent and efferent arteriolar tone is not specifically known. Also not known is whether TRV120027 affects podocyte function and the filtration coefficient. Of note, losartan in an ovine model of HF also maintained GFR and urinary sodium excretion despite a reduction in renal perfusion pressure. To better characterize tubular sodium handling we used the lithium clearance technique to assess proximal and distal fractional reabsorption of sodium. During drug administration, sodium reabsorption was not different.
between groups even though TRV120027 reduced renal perfusion pressure, an effect that would be expected to increase sodium reabsorption. The preserved sodium excretion can be explained with TRV120027 blocking the tubular actions of angiotensin II, which is an important mediator of increased sodium reabsorption when renal perfusion pressure is decreased. Interestingly, in the post-infusion clearance distal fractional sodium reabsorption was significantly lower in the furosemide+TRV120027 group. This could be explained by aldosterone, plasma levels of which tended to be reduced with TRV120027 during C3. Aldosterone acts via the mineralocorticoid receptor to promote sodium reabsorption in the distal tubule. Thus, aldosterone-reducing actions of TRV120027 may be of special importance as aldosterone affects nephron segments at which loop diuretics do not act. While the clinical impact of these findings remains to be proven, these data indicate that TRV120027 has the potential to be renal enhancing or protective.

A hallmark of HF is neurohumoral activation. Increases of angiotensin II and aldosterone, if not antagonized, can promote sodium retention, vasoconstriction, and organ fibrosis, which all can contribute to the progression of HF. As seen with angiotensin receptor blockers, plasma renin activity significantly increased with addition of TRV120027. This could in part be due to the reduction in renal perfusion pressure but also due to antagonizing the inhibitory action of angiotensin II on renin secretion by TRV120027. While there was a trend for angiotensin II to increase in the furosemide alone group, we were not able to assess the angiotensin II levels in the furosemide+TRV120027 group as the angiotensin II assay cross-reacts with TRV120027. Importantly, given that aldosterone, the secretion of which is stimulated by angiotensin II, decreased with TRV120027 suggests that downstream signaling of renin was effectively blocked, which would mean that the increase of plasma renin activity with TRV120027 would be of little consequence.
In summary, TRV120027 when added to furosemide unloaded the heart, preserved renal function during drug administration, and led to a more sustained diuresis post-infusion, which may be due to aldosterone suppression. These findings suggest that TRV120027 may be a promising new drug for the treatment of ADHF and further studies are warranted.

Disclosures

Guido Boerrigter and John C. Burnett, Jr received research support from Trevena, Inc. Michael Lark, David Soergel, and Jonathan Violin are employed by Trevena, Inc.

References


Table. Cardiorenal and humoral function with furosemide+vehicle vs. furosemide+TRV120027.

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<td>Furosemide + TRV120027</td>
<td>18 (9/27)</td>
<td>30 (26/36)</td>
<td>35 (26/45)†</td>
<td>19 (15/33)</td>
</tr>
<tr>
<td><strong>Angiotensin II, pg/mL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide + Vehicle</td>
<td>101 (67/137)</td>
<td>114 (70/170)</td>
<td>116 (69/130)</td>
<td>86 (63/110)</td>
</tr>
<tr>
<td>Furosemide + TRV120027</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>B-type natriuretic peptide, pg/mL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide + Vehicle</td>
<td>45±18</td>
<td>54±14</td>
<td>44±14</td>
<td>50±13</td>
</tr>
<tr>
<td>Furosemide + TRV120027</td>
<td>47±31</td>
<td>46±21</td>
<td>38±13</td>
<td>36±17</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation or median (25th/75th percentile). *p<0.05 vs. respective baseline. †p<0.05 between groups for change from baseline. NA, not available due to crossreactivity of TRV120027 with assay; PFRNa, proximal fractional reabsorption of sodium.
Figure Legends

**Figure 1.** Hemodynamic actions of furosemide + saline (black lines) and furosemide + TRV120027 (red lines) in canine experimental heart failure. Changes from baseline (C1) are shown for mean arterial pressure (A), cardiac output (B), systemic vascular resistance (C), pulmonary capillary wedge pressure (D), and pulmonary vascular resistance. Values are mean ± standard error of the mean. *p<0.05 vs. C1 in the furosemide + saline group; †p<0.05 vs. C1 in the furosemide + TRV120027 group; #p<0.05 between groups. C1, baseline clearance; C2, first drug infusion clearance (furosemide 1 mg/kg/h; TRV120027 0.3 μg/kg/min); C3, second drug infusion clearance (furosemide 1 mg/kg/h; TRV120027 1.5 μg/kg/min); C4, post-infusion clearance.

**Figure 2.** Hemodynamic actions of furosemide + saline (black lines) and furosemide + TRV120027 (red lines) in canine experimental heart failure. Changes from baseline (C1) are shown for urine flow (A), urinary sodium excretion (B), distal fractional sodium reabsorption (C), plasma aldosterone (D), and atrial natriuretic peptide. Values are mean ± standard error of the mean. *p<0.05 vs. C1 in the furosemide + saline group; †p<0.05 vs. C1 in the furosemide + TRV120027 group; #p<0.05 between groups. C1, baseline clearance; C2, first drug infusion clearance (furosemide 1 mg/kg/h; TRV120027 0.3 μg/kg/min); C3, second drug infusion clearance (furosemide 1 mg/kg/h; TRV120027 1.5 μg/kg/min); C4, post-infusion clearance.
Figure 1.

A. △ Mean arterial pressure

B. △ Cardiac output

C. △ Systemic vascular resistance

D. △ Pulmonary vascular resistance

E. △ Pulmonary capillary wedge pressure
Figure 2.

A. Δ Urine flow

B. Δ Urinary sodium excretion

C. Δ Distal Fractional Na⁺-Reabsorption

D. Δ Aldosterone

E. Δ Atrial natriuretic peptide