Cardiorenal Actions of TRV120027, a Novel β-Arrestin–Biased Ligand at the Angiotensin II Type I Receptor, in Healthy and Heart Failure Canines
A Novel Therapeutic Strategy for Acute Heart Failure

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Background—The angiotensin II type 1 receptor (AT1R) plays a key role in regulating cardiorenal function. Classic “unbiased” AT1R antagonists block receptor coupling to both \( \frac{G}{H9251} \) and β-arrestin–mediated signals, which desensitize G-protein signaling as well as transduce G-protein–independent signals. TRV120027 is a novel β-arrestin–biased AT1R ligand, which engages β-arrestins while blocking G-protein signaling. At the AT1R, TRV120027 can inhibit angiotensin II–mediated vasoconstriction, whereas, through β-arrestin coupling, increase cardiomyocyte contractility. We defined for the first time the acute cardiorenal actions of TRV120027 in healthy and heart failure (HF) canines.

Methods and Results—Healthy and HF canines (induced by tachypacing) were anesthetized. After instrumentation and equilibration, a 30-minute baseline clearance was performed, followed by further clearance with escalating doses of intravenous TRV120027 (0.01, 0.1, 1, 10, and 100 \( \mu g/kg \) per minute) and a 30-minute washout. In healthy canines, TRV120027 decreased pulmonary capillary wedge pressure and systemic and renal vascular resistances, while increasing cardiac output, renal blood flow, glomerular filtration rate, and urinary sodium excretion. In HF canines, TRV120027 decreased mean arterial pressure, right atrial pressure, and pulmonary capillary wedge pressure, systemic and renal vascular resistances and increased cardiac output and renal blood flow. Glomerular filtration rate and urinary sodium excretion were maintained.

Conclusions—We report for the first time the cardiorenal actions of the novel β-arrestin–biased AT1R ligand TRV120027. In both normal and HF canines, TRV120027 demonstrated cardiac unloading actions while preserving renal function. With this beneficial pharmacological profile, TRV120027 represents a novel strategy for the treatment of HF. (Circ Heart Fail. 2011;4:770-778.)

Key Words: angiotensin II receptors heart failure drugs pharmacology

Heart failure (HF) continues to be associated with high morbidity and mortality, and there is an urgent need for new therapeutic strategies. A major target of currently available drugs is the superfamily of 7-transmembrane receptors (7TMRs), which, due to their association with heterotrimeric G proteins, are also referred to as G-protein–coupled receptors (GPCRs). The current understanding of their signaling mechanisms is that agonist binding to the extracellular domain leads to intracellular dissociation of a heterotrimeric G protein into \( G_{\alpha} \) and \( G_{\beta \gamma} \) subunits, resulting in activation of second messenger mediated cellular responses. A second set of proteins, the β-arrestins, were first described to desensitize GPCRs and promote receptor internalization. More recently, β-arrestins were shown also to activate specific signaling pathways in a G-protein–independent manner and drive biology which is distinct from G-protein–mediated cellular responses. Previous studies have shown that some ligands can selectively activate either G-protein or β-arrestin pathways, for example, they can be biased toward activating one pathway over the other. Rather than indiscriminately blocking or activating all receptor-mediated functions, as occurs with unbiased ligands, biased ligands selectively engage a subset of the pathways downstream of the receptor. Thus, in comparison to classic unbiased ligands, biased ligands have the potential to more selectively target desired beneficial signaling. Importantly, a novel β-arrestin–biased ligand for the angiotensin II type 1 (AT1R), TRV120027, was recently reported and is currently in development for the treatment of acute decompensated heart failure (HF). TRV120027 antagonizes G-protein signaling.
in the same manner as conventional angiotensin receptor blockers (ARBs), but, unlike ARBs, it stimulates β-arrrestin recruitment and consequent downstream signals. In rodents, this ligand showed a unique cardiovascular profile: it had vasodilating effects similar to a conventional ARB, but, unlike the ARB, also enhanced cardiac contractility while decreasing myocardial oxygen consumption as assessed by ventricular systolic pressure-volume area.13

Clinical Perspective on p 778

Neurohumoral activation including activation of the renin-angiotensin-aldosterone system (RAAS) is a central mechanism driving progression of HF. Indeed, pharmacological inhibition of the RAAS with ACE inhibitors, ARBs, and mineralocorticoid receptor antagonists are among the more effective therapies for HF.14,15 Thus, the unique pharmacological profile of TRV120027 could represent an improved therapeutic agent for the treatment of HF.

The goal of this study was to characterize for the first time the cardiorenal and humoral actions of TRV120027 in healthy canines and canines with experimental HF. We hypothesized that TRV120027 would have beneficial cardiorenal actions that could translate into a novel effective therapy for the treatment of acute decompensated HF.

Methods

These studies were performed in accordance with the Animal Welfare Act and were approved by the Mayo Clinic Animal Care and Use Committee. The studies were done under general anesthesia in 3 groups of male mongrel dogs (body weight, 21.5–29.0 kg), 1 group healthy, the other 2 with pacing-induced HF, with methodologies as previously reported.16–19 Dogs were maintained on a sodium controlled diet (Hill’s I/d diet). TRV120027 was kindly provided by Trevena, Inc, King of Prussia, PA. In prior studies in healthy male beagles, TRV120027 achieved steady state within 15 minutes of continuous intravenous dosing and had a half-life of approximately 2 minutes.

Studies in Healthy Canines

The day before the acute experiment, animals (n=4) were fed 300 mg of lithium carbonate for the assessment of renal tubular function and were fasted with ad libitum access to water. On the day of the study, animals were anesthetized with intravenous fentanyl and pentobarbital, intubated, and mechanically ventilated with room air and supplemental oxygen (3 L/min). A flow-directed balloon-tipped thermodilution catheter was inserted through the right external jugular vein for hemodynamic measurements, and aortic pressure was assessed by means of a line inserted through the femoral artery. Cardiac output was assessed by the thermodilution method in triplicate and averaged (Cardiac output model 9510-A computer, American Edwards Laboratories, Irvine, CA). Through a left lateral flank incision, the left ureter was cannulated for urine collection. An electromagnetic flow probe was placed on the renal artery (Carolina Medical Electronics) to measure renal blood flow. All renal parameters reported are for the left kidney only. After surgical preparation, inulin (1 mL/min; preceded by a weight-adjusted bolus) and saline (1 mL/min) were continuously administered through lines in the femoral vein. After 60 minutes of equilibration, a 30-minute baseline clearance was done that included urine collection, blood sampling (taken midway through the clearance), and hemodynamic measurements. Pressure tracings and renal blood flow were recorded and analyzed digitally (Sonometrics Corporation, London, Ontario, Canada). After the baseline clearance, the saline infusion was replaced with a first dose of TRV120027 (0.01 µg/kg per minute with an infusion rate of 1 mL/min). After a lead-in period of 15 minutes, a 20-minute clearance was done. Then, TRV120027 was infused for 20 minutes at 0.1 µg/kg per minute (infusion rate, 1 mL/min), during which another clearance was performed. In like fashion, 20-minute clearances with 1.0 and 10.0 µg/kg per minute were done, which were followed by a 30-minute clearance with 100 µg/kg per minute. All drug infusions were done with an infusion rate of 1 mL/min. After the final dose of TRV120027, infusion of the study drug was replaced with saline (1 mL/min), and after a 30-minute washout, a final 30-minute clearance was done. The drug doses were chosen on the basis of initial dose-finding studies (data not shown). In normal canines, plasma levels of TRV120027 increases linearly with dose (0.1–100 µg/kg per minute) and of particular note, plasma levels of TRV120027 were 22.2±1.9 ng/mL after 45 minutes of dosing with 1 µg/kg per minute and 154±45 ng/mL after 45 minutes of dosing with 10 µg/kg per minute. In a second normal canine study, steady-state plasma levels (252±42 ng/mL) were reached within 15 minutes of dosing of 10 µg/kg per minute of TRV120027. In the first study, the T1/2 after termination of dosing was 0.2±0.03 hours and in the second it was 0.301±0.001 hours, suggesting that TRV120027 is rapidly eliminated in normal canines. Of note, only the first clearance period had a 15-minute lead-in period, and the final dose level (dose 5) was of longer duration (30 minutes) than the earlier 4 periods; therefore, the duration of time at steady state during the first and last clearance periods were longer than in the other clearance periods. However, the aim of this study was to assess the actions of a wide dose range of TRV120027 and not to specifically assess the precise pharmaco-kinetic/pharmacodynamic relationship; therefore, the study was designed to allow for optimal evaluation of the pharmacology over a wide range of doses, with a view to limiting the total length of time the animals were anesthetized.

Studies in Experimental HF

Severe HF was induced in 6 dogs by rapid right ventricular pacing at 240 beats per minute, as previously described in detail.17 On day 11 of pacing, an acute study was done as described above for the healthy animals with the sole difference that all clearances lasted 30 minutes to allow sufficient time for urine collection despite the sodium and water retention in this model. Pacing was suspended for the time of surgical preparation but restarted before the equilibration period and continued throughout the acute protocol.

Another group of HF dogs (n=4) was used to assess the reversibility of the actions of TRV120027. Pacing protocol and surgical setup were the same as above. After the 30-minute baseline clearance, TRV120027 (1.0 µg/kg per minute) was infused for 45 minutes and then stopped. Mean arterial pressure was measured at 5-minute intervals before, during, and for 180 minutes after TRV120027 infusion. Blood samples were collected on ice in EDTA tubes at 30 and 40 minutes into the drug infusion and 3, 6, 15, 30, 60, 120, and 180 minutes after the end of drug infusion. After centrifugation of the blood samples, 0.5 mL of plasma was combined with 0.5 mL of 1N acetic acid, and the samples were stored at −80°C until shipment on dry ice to Absorption Systems, LP, Exton, PA, for measurement of drug concentrations.

Laboratory Analyses

Electrolytes were measured by flame photometry (IL943, Instrumentation Laboratory, Lexington, MA). Glomerular filtration rate (GFR) was calculated by inulin clearance. Plasma and urine inulin were measured by the anthrone method.20 Proximal and distal fractional sodium reabsorption were assessed with the lithium clearance technique. Proximal fractional reabsorption of sodium (in percent) was calculated as (1−[lithium clearance/GFR])×100. Distal fractional reabsorption of sodium (in percent) was calculated as:

\[\text{DFR}(\%\text{F}) = \left(\frac{[\text{lithium clearance minus sodium clearance}]}{\text{lithium clearance}}\right) \times 100\]

Filtration fraction was calculated as GFR divided by (renal blood flow×[1−hematocrit]). Plasma renin activity, angiotensin II, aldosterone, atrial and B-type natriuretic peptide (ANP and BNP, respectively) were measured by radioimmunoassay as described previously.16 As the angiotensin II assay detected TRV120027 to a significant degree, only baseline levels of angiotensin II were reported. Plasma catecholamines were measured in the Immunochemical Core Laboratory of the Mayo Clinic, Rochester.
Table 1. Cardiorenal and Humoral Functions With 5 Doses of TRV120027 in Healthy Anesthetized Canines (n=4)

<table>
<thead>
<tr>
<th>Hemodynamic function</th>
<th>Baseline</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>Post</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>1.2±0.4</td>
<td>1.1±0.4</td>
<td>1.1±0.4</td>
<td>1.2±0.4</td>
<td>0.9±0.4</td>
<td>0.8±0.4</td>
<td>0.6±0.4</td>
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<tr>
<td>Pulmonary artery pressure, mm Hg</td>
<td>13.6±1.2</td>
<td>13.5±1.2</td>
<td>13.2±1.0</td>
<td>13.2±1.0</td>
<td>13.1±0.9</td>
<td>13.0±0.7</td>
<td>12.9±0.5</td>
<td>0.0513</td>
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<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>4.9±0.6</td>
<td>4.2±0.5</td>
<td>4.1±0.4</td>
<td>4.2±0.5</td>
<td>3.5±0.6</td>
<td>3.3±0.6</td>
<td>2.9±0.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>113±16</td>
<td>120±16</td>
<td>118±16</td>
<td>119±15</td>
<td>126±17</td>
<td>132±16</td>
<td>138±15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>30±2</td>
<td>30±2</td>
<td>31±2</td>
<td>31±2</td>
<td>32±3</td>
<td>30±3</td>
<td>30±3</td>
<td>0.16</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, mm Hg · L⁻¹ · min⁻¹</td>
<td>2.7±0.3</td>
<td>2.6±0.3</td>
<td>2.6±0.2</td>
<td>2.5±0.3</td>
<td>2.4±0.2</td>
<td>2.5±0.2</td>
<td>2.5±0.3</td>
<td>0.016</td>
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<tr>
<td>Renal perfusion pressure, mm Hg</td>
<td>125±3</td>
<td>126±5</td>
<td>125±4</td>
<td>125±4</td>
<td>122±5</td>
<td>120±4</td>
<td>124±4</td>
<td>0.0134</td>
</tr>
<tr>
<td>LV external work, J/min</td>
<td>52±5</td>
<td>57±7</td>
<td>57±7</td>
<td>57±5</td>
<td>60±6</td>
<td>59±6</td>
<td>64±6</td>
<td>0.0035</td>
</tr>
</tbody>
</table>

| Renal function | Glomerular filtration rate, mL/min | 28±1 | 29±1 | 28±3 | 44±8 | 38±2 | 40±3 | 42±2 | 0.0041 |
| Urine flow, mL/min | 0.10±0.01 | 0.10±0.02 | 0.12±0.02 | 0.19±0.05 | 0.19±0.04 | 0.23±0.05 | 0.27±0.06 | <0.0001 |
| Urinary Na⁺ excretion, μEq/min | 7±2 | 10±3 | 15±4 | 29±7 | 36±9 | 49±14 | 60±14 | 0.0002 |
| Urinary K⁺ excretion, μEq/min | 9±2 | 9±2 | 11±2 | 18±7 | 18±5 | 22±2 | 29±4 | 0.0005 |
| Filtration fraction | 0.22±0.02 | 0.22±0.02 | 0.20±0.02 | 0.29±0.05 | 0.23±0.02 | 0.22±0.02 | 0.22±0.03 | 0.52 |
| Prox. fractional Na⁺ reabsorption, % | 86±2 | 84±4 | 79±5 | 80±4 | 77±5 | 73±5 | 69±6 | <0.0001 |
| Distal fractional Na⁺ reabsorption, % | 98.3±6 | 98.3±6 | 98.0±4 | 97.4±0.6 | 97.0±0.6 | 96.9±0.6 | 96.7±0.3 | 0.003 |

| Humoral function | Plasma Na⁺, mmol/L | 141±2 | 137±2 | 138±2 | 139±2 | 138±3 | 139±2 | 139±2 | 0.83 |
| Plasma K⁺, mmol/L | 4.4±0.2 | 4.1±0.1 | 4.1±0.1 | 4.2±0.2 | 4.1±0.1 | 4.1±0.1 | 4.1±0.1 | 0.04 |
| Hematocrit, % | 37±1 | 37±1 | 36±1 | 35±1 | 35±1 | 36±1 | 36±1 | 0.0029 |
| Aldosterone, ng/dL | 4.1±1.3 | 4.1±1.2 | 4.4±1.0 | 5.9±1.7 | 3.5±0.4 | 2.8±0.3 | 3.7±0.4 | 0.2 |
| Atrial natriuretic peptide, pg/mL | 31±2 | 35±7 | 36±10 | 35±8 | 38±9 | 31±7 | 33±7 | 0.73 |
| B-type natriuretic peptide, pg/mL | 17±7 | 12±3 | 10±1 | 10±2 | 11±3 | 11±2 | 11±2 | 0.28 |
| Cyclic GMP, pmol/mL | 9.8±1.1 | 9.1±1.0 | 8.7±0.7 | 7.1±1.2 | 6.7±1.0 | 7.4±0.8 | 5.9±0.4 | 0.0048 |
| Norepinephrine, pg/mL | 117±19 | 112±18 | 113±14 | 128±29 | 134±21 | 166±39 | 172±38 | 0.0049 |
| Epinephrine, pg/mL | 14±4 | 12±2 | 12±2 | 12±2 | 10±0 | 13±3 | 22±9 | 0.27 |
| Dopamine, pg/mL | 22±11 | 21±6 | 18±5 | 19±4 | 25±13 | 56±26 | 24±6 | 0.09 |

Post indicates postinfusion clearances; LV, left ventricular.
*P value from linear trend test. TRV120027 doses infused during the drug infusion clearances were D1, 0.01 μg/kg per minute; D2, 0.1 μg/kg per minute; D3, 1.0 μg/kg per minute; D4, 10.0 μg/kg per minute; and D5, 100 μg/kg per minute.

MN.²¹ Renal perfusion pressure was calculated as mean arterial pressure minus right atrial pressure. Left ventricular external work (J/min) was estimated as (mean arterial pressure-pulmonary capillary wedge pressure)×cardiac output ÷0.13.

Plasma concentrations of TRV120027 were measured by Absorption Systems, LP (Exton, PA) using an approach that included acetoni-trile precipitation, high-pressure liquid chromatography, and mass spectrometry. The lower limit of detection of this assay was 5 ng/mL.

Statistical Analysis
Values are expressed as mean±SEM. Drug effect was tested by linear trend analysis for repeated measurements, for which the 6 clearances from baseline through the highest dose of TRV120027 were considered; values during the postinfusion clearance are reported but were not included in the statistical analysis. A linear trend analysis was chosen to take advantage of the ordinal nature of the data. Of note, in the case of a linear pattern, an ANOVA that does not take into account the linear nature of the data would have less power because it would try to show that the 6 clearances have different mean levels, resulting in a 5 degrees of freedom test in which deviations from the group means have to be quite large to be significant as the test has to allow for the possibility of random deviations of the group means in a 5-dimensional space. In contrast, the linear trend test requires the means to deviate not randomly in any direction in this 5-dimensional space but rather in a linear pattern, thus focusing power on the linear deviations at the expense of lower power for the nonlinear deviations. In the reversibility study, mean arterial pressure during and after drug administration was compared with baseline with 1-way ANOVA for repeated measures with post hoc Dunnett test. A probability value <0.05 was considered statistically significant. Statistical analyses were performed with GraphPad Prism, Version 5.02 for Windows (GraphPad Software, Inc, La Jolla, CA).

Results

Studies in Healthy Canines
Results are shown in Table 1 and Figure 1.

Hemodynamic Function
In normal canines, TRV120027 decreased mean arterial pressure and systemic vascular resistance while increasing cardiac output (Figure 1A through 1C). Similarly, renal blood flow increased, whereas renal vascular resistance decreased.
(Figure 1D and 1E). Right atrial pressure and pulmonary capillary wedge pressure decreased, as did renal perfusion pressure and pulmonary vascular resistance, whereas pulmonary artery pressure tended to decrease. Heart rate and left ventricular external work increased, whereas stroke volume remained unchanged.

Renal Function
TRV120027 significantly increased GFR, whereas filtration fraction was unchanged. Urine flow and urinary sodium and potassium excretion increased, whereas proximal and distal fractional sodium excretion decreased.

Humoral Function
Plasma sodium remained unchanged, whereas plasma potassium decreased. Plasma renin activity increased with administration of TRV120027 (Figure 1F), whereas aldosterone levels were unchanged. The increase of plasma renin activity probably is due to the study drug, which competes with angiotensin II at the AT1R and thus interrupts a feedback loop. Plasma ANP and BNP levels were unchanged, whereas plasma cGMP decreased. Hematocrit decreased. Plasma norepinephrine increased, whereas epinephrine and dopamine did not significantly change (of note, the lower limit of detection for both epinephrine and dopamine is 10 pg/mL; samples with values below the lower limit of detection were set as 10 pg/mL).

Studies in Experimental HF
Results are shown in Table 2 and Figure 2. Compared with normal canines, HF was associated with significantly increased cardiac filling pressures and systemic vascular resistance, reduced cardiac output and renal blood flow, increased filtration fraction, reduced urinary sodium excretion, and activation of several neurohumoral systems. Plasma levels of angiotensin II were elevated in HF compared with normal canines (108±35 pg/mL versus 10±3 pg/mL, P<0.03).

Hemodynamic Function
TRV120027 administration in experimental HF reduced mean arterial pressure (Figure 2A), right atrial pressure, pulmonary artery pressure, pulmonary capillary wedge pressure (Figure 2B), and renal perfusion pressure. Systemic and renal vascular resistance decreased, whereas cardiac output (Figure 2C) and renal blood flow (Figure 2D) increased. Left ventricular external work decreased. As pacing was continued throughout the drug infusion protocol, the effect of TRV120027 on heart rate could not be assessed.
Table 2. Cardiorenal and Humoral Functions With 5 Doses of TRV120027 in Canines With Experimental Heart Failure (n=6)

<table>
<thead>
<tr>
<th>Hemodynamic function</th>
<th>Baseline</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>Post</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>10.2±1.9</td>
<td>9.8±1.8</td>
<td>9.7±1.8</td>
<td>9.2±1.7</td>
<td>8.6±1.4</td>
<td>8.6±1.5</td>
<td>8.5±1.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulmonary artery pressure, mm Hg</td>
<td>30.1±2.5</td>
<td>28.8±2.3</td>
<td>27.9±2.1</td>
<td>25.4±1.9</td>
<td>24.1±1.7</td>
<td>23.6±1.8</td>
<td>23.7±1.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systemic vascular resistance, mm Hg · L⁻¹ · min⁻¹</td>
<td>62±7</td>
<td>59±7</td>
<td>53±3</td>
<td>46±4</td>
<td>43±3</td>
<td>41±4</td>
<td>46±5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, mm Hg · L⁻¹ · min⁻¹</td>
<td>4.2±0.7</td>
<td>3.9±0.8</td>
<td>4.1±0.8</td>
<td>3.8±0.6</td>
<td>3.9±0.6</td>
<td>4.0±0.7</td>
<td>4.3±0.8</td>
<td>0.31</td>
</tr>
<tr>
<td>Renal vascular resistance, mm Hg · L⁻¹ · min⁻¹</td>
<td>792±135</td>
<td>698±93</td>
<td>602±99</td>
<td>475±94</td>
<td>452±109</td>
<td>478±172</td>
<td>522±227</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Renal perfusion pressure, mm Hg</td>
<td>103±7</td>
<td>99±7</td>
<td>95±6</td>
<td>84±6</td>
<td>80±5</td>
<td>78±4</td>
<td>80±4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LV external work, J/min</td>
<td>21±3</td>
<td>20±3</td>
<td>20±2</td>
<td>19±2</td>
<td>18±2</td>
<td>18±3</td>
<td>17±2</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

Renal function

| Glomerular filtration rate, mL/min | 27±2 | 31±4 | 30±3 | 29±4 | 28±7 | 27±7 | 29±7 | 0.65 |
| Urine flow, mL/min | 0.11±0.01 | 0.13±0.01 | 0.13±0.01 | 0.12±0.02 | 0.13±0.03 | 0.13±0.03 | 0.22±0.09 | 0.62 |
| Urinary sodium excretion, μEq/min | 3.4±0.9 | 4.8±1.5 | 4.5±1.3 | 4.7±1.5 | 8.4±3.8 | 14.3±7.9 | 38.5±25.3 | 0.02 |
| Urinary potassium excretion, μEq/min | 15.6±2.7 | 19.8±3.6 | 19.2±3.2 | 20.6±5.9 | 24.3±8.5 | 25.7±9.3 | 31.6±7.8 | 0.023 |
| Prox. fractional Na⁺ reabsorption, % | 91±2 | 88±3 | 86±4 | 85±5 | 83±6 | 81±8 | 75±10 | 0.0038 |
| Distal fractional Na⁺ reabsorption, % | 99.0±0.3 | 99.3±0.1 | 99.4±0.1 | 99.3±0.2 | 99.0±0.2 | 98.5±0.3 | 98.3±0.6 | 0.0065 |

Humoral function

| Plasma Na⁺, mmol/L | 146±3 | 145±2 | 146±3 | 145±2 | 145±2 | 144±2 | 144±3 | 0.27 |
| Plasma K⁺, mmol/L | 4.9±0.2 | 4.5±0.1 | 4.5±0.1 | 4.5±0.1 | 4.5±0.2 | 4.5±0.2 | 4.6±0.2 | 0.012 |
| Hematocrit, % | 44±4 | 44±4 | 43±4 | 43±4 | 43±4 | 41±4 | 42±4 | 0.0005 |
| Plasma renin activity, ng · mL⁻¹ · h⁻¹ | 25±7 | 22±7 | 19±5 | 23±7 | 27±6 | 27±7 | 19±6 | 0.30 |
| Atrial natriuretic peptide, pg/mL | 783±112 | 733±121 | 731±105 | 585±96 | 779±162 | 929±172 | 577±89 | 0.20 |
| B-type natriuretic peptide, pg/mL | 46±14 | 52±13 | 49±12 | 48±12 | 41±12 | 43±10 | 38±10 | 0.10 |
| Cyclic GMP, pmol/mL | 23±1 | 21±2 | 20±2 | 17±2 | 17±2 | 15±1 | 14±2 | <0.0001 |
| Norepinephrine, pg/mL | 785±92 | 735±75 | 700±110 | 661±71 | 735±131 | 760±141 | 748±97 | 0.64 |
| Epinephrine, pg/mL | 86±30 | 42±6 | 33±6 | 33±9 | 29±10 | 36±13 | 68±29 | 0.017 |
| Dopamine, pg/mL | 126±34 | 146±45 | 142±31 | 165±35 | 160±31 | 167±32 | 195±52 | 0.002 |

Post indicates postinfusion clearances; LV, left ventricular.

*P value from linear trend test. TRV120027 doses infused during the drug infusion clearances were D1, 0.01 μg/kg per minute; D2, 0.1 μg/kg per minute; D3, 1.0 μg/kg per minute; D4, 10.0 μg/kg per minute; and D5, 100 μg/kg per minute.

Renal Function

Despite its blood pressure–lowering actions, TRV120027 preserved GFR. Filtration fraction decreased (Figure 2E). Urine flow remained unchanged, whereas urinary sodium and potassium excretion increased. Proximal and distal fractional sodium excretion decreased.

Humoral Function

Plasma sodium remained unchanged, whereas plasma potassium decreased. Aldosterone decreased with TRV120027 (Figure 2F), whereas plasma renin activity remained unchanged. Hematocrit decreased slightly. There were no significant changes in norepinephrine, whereas epinephrine decreased and dopamine increased. ANP and BNP were unchanged, but cyclic guanosine monophosphate (cGMP) decreased.

Reversibility Study in Experimental HF

Figure 3 shows mean arterial pressure in canines with HF before, during, and after a 45-minute infusion of TRV120027 at 1 μg/kg per minute. This infusion yielded a mean plasma level of TRV120027 of 13.6±8.6 ng/mL at 30 minutes and 14.1±7.2 ng/mL at 40 minutes after initiation of dosing, suggesting that steady state had been achieved. These plasma levels were similar to those seen in normal canines after 45 minutes of infusion (22.2±1.9 ng/mL). Within 6 minutes after termination of dosing, 3 of the 4 animals had plasma levels less than the lower limit of quantification of 5 ng/mL (the fourth animal had a blood level of 5.44 ng/mL), consistent with the rapid clearance seen in normal animals. Mean arterial pressure was significantly reduced compared with baseline, starting 10 minutes after the start of the infusion and remained so for the remainder of the drug infusion. After discontinuation of TRV120027, mean arterial pressure quickly increased and was reduced compared with baseline for only the first 10 minutes. The rapid reversal of mean arterial pressure is consistent with the approximately 2-minute half-life of TRV120027 in canines after infusion to steady-state exposure.
We report for the first time the cardiorenal actions of TRV120027, a β-arrestin–biased AT1R ligand, which, like ARBs, antagonizes G-protein–coupled signaling, but, unlike ARBs, activates β-arrestin signaling. In both normal and HF canines, TRV120027 has cardiac unloading actions while preserving renal function. The compound rapidly achieves steady state and has a short systemic half-life. The pharmacokinetic features are consistent with pharmacological titratability of the agent and should allow individualization of therapy. With this beneficial profile, TRV120027 represents a novel therapeutic strategy for patients with HF, and, with its rapid onset of action as well as reversibility, the compound could be particularly well suited for the treatment of patients with acute decompensated HF.

TRV120027 belongs to a new class of pharmacological agents, specifically β-arrestin–biased AT1R ligands. In previous studies, TRV120027 behaved as a vasodilator consistent with angiotensin II antagonism while increasing cardiac performance.13 Violin et al reported that TRV120027, similar to ARBs, blocked AT1R-mediated G-protein activation as measured by IP1 accumulation, but, in contrast to ARBs, TRV120027 stimulated β-arrestin recruitment to the AT1R. Furthermore, TRV120027 activated a unique pattern of downstream signaling, including the ERK1/2 MAPKs, src, and eNOS, phospho-FAK, and phospho-c-Jun consistent with β-arrestin recruitment.13 Importantly, our study is the first to assess how TRV120027, with its unique mechanism of action, could be particularly well suited for the treatment of patients with acute decompensated HF.

Figure 2. TRV120027 in canines with experimental heart failure (n=6). Effect of TRV120027 on mean arterial pressure (A), pulmonary capillary wedge pressure (B), cardiac output (C), renal blood flow (D), filtration fraction (E), and plasma aldosterone (F) are shown. Graphs show changes from baseline. Baseline values were mean arterial pressure, 114±8 mm Hg; pulmonary capillary wedge pressure, 23±2 mm Hg · L⁻¹ · min⁻¹; cardiac output, 1.7±0.2 L/min; renal blood flow, 141±44 mL/min; filtration fraction, 0.40±0.07 mm Hg · L⁻¹ · min⁻¹; and aldosterone, 37±15 ng · mL⁻¹ · h⁻¹. Probability values are for linear trend. Doses of TRV120027 infused during the clearances were D1, 0.01 μg/kg per minute; D2, 0.1 μg/kg per minute; D3, 1.0 μg/kg per minute; D4, 10.0 μg/kg per minute; and D5, 100 μg/kg per minute. Post indicates postinfusion clearance.

Figure 3. Time course of mean arterial pressure before, during, and after a 75-minute infusion of TRV120027 (1 μg/kg per minute) in canines with experimental heart failure (n=4). Mean arterial blood pressure was measured every 5 minutes. *P<0.05 versus baseline average with 1-way ANOVA for repeated measurements with post hoc Dunnett test.
action, affects not only cardiovascular but also renal and humoral function, in both healthy and HF canines. TRV120027 reduced systemic and renal vascular resistances in both healthy and HF canines, which resulted in significant increases in renal blood flow and cardiac output. Mean arterial pressure decreased in both groups but to a lesser degree in the healthy group, which could be due to sympathetic activation as evidenced by an increase in heart rate and plasma norepinephrine. The larger reduction in mean arterial pressure in the HF group could also be due to higher activation of the RAAS at baseline, consistent with the relatively larger reduction in systemic vascular resistance with TRV120027 in this group. Right atrial and pulmonary capillary wedge pressure decreased in both groups, which could be due to the reduction in afterload but could also indicate a venodilating effect of TRV120027. TRV120027 has been shown to increase cardiac contractility in isolated murine cardiomyocytes and in anesthetized rats, as indicated by a dose-dependent increase in the slope of the end-systolic pressure-volume relationship. In the current HF canine study, cardiac output increased with TRV120027, consistent with the observations made in normal rats and the direct effect of the compound on cardiomyocyte contractility. The moderate effect on cardiac output in HF canines may reflect the mechanistically distinct and generally more modest effect of AT1R-biased ligands on increasing cardiomyocyte contractility compared with classic inotropes. Of note, Chan et al using a similar experimental setup reported that the unbiased AT1R antagonist losartan in healthy, anesthetized canines decreased renal vascular resistance and increased renal blood flow but importantly, did not change mean arterial pressure, heart rate, cardiac output, or systemic vascular resistance; no significant changes were reported for a time control group with vehicle infusion.

Although renal blood flow increased and renal vascular resistance decreased with TRV120027, urine flow and GFR increased in the healthy group. Potential mechanisms for the increased GFR include differential actions of TRV120027 on afferent and efferent arterioles, resulting in an increased intraglomerular hydrostatic pressure, inhibition of tubuloglomerular feedback, and an increase in the ultrafiltration coefficient. Urinary sodium excretion increased, which was associated with decreased proximal and distal fractional sodium reabsorption. Also, urinary potassium increased, which may be due to the increased urine flow. Filtration fraction remained unchanged with TRV120027. Of note, filtration fraction was in the normal range at baseline, consistent with little action of endogenous angiotensin II on the efferent arteriole of the glomerulus, and therefore little effect of AT1R blockade would be expected. These renal actions, are similar to those reported by Chan et al for losartan in healthy canines. Together, the cardiorenal effects of TRV120027 suggest that this β-arrestin-biased AT1R ligand shares some features of ARBs, such as renal effects, but is unique in others, such as the hemodynamic effects. This is consistent with head-to-head comparison of TRV120027 to ARBs in vitro and in normal rats.

In the HF group, GFR and urine flow were maintained, whereas urinary sodium excretion increased slightly, despite the reduction in renal perfusion pressure. A possible explanation is that although a reduced perfusion pressure would reduce the glomerular hydrostatic pressure and thus filtration, an increased renal blood flow at the same GFR should reduce the increase in oncotic pressure that occurs along the glomerulus with filtration and that opposes filtration. Indeed, filtration fraction decreased, which is consistent with TRV120027 antagonizing the angiotensin II–mediated vasoconstriction at the efferent arteriole of the glomerulus. The reduced oncotic pressure in the postglomerular capillaries would also be expected to reduce reabsorption from the tubule. Because renal oxygen delivery depends on renal blood flow and renal oxygen consumption is primarily determined by sodium reabsorption, which correlates well with GFR as most of the filtered sodium in the tubule is reabsorbed, an increase of renal blood flow relative to GFR could improve renal oxygen status.

In healthy canines, TRV120027 dose-dependently increased plasma renin activity, consistent with AT1R blockade. In HF canines, plasma renin activity was increased compared with the normal group at baseline and remained unchanged, which may be due to already high activation of this system at baseline, as also indicated by the elevated angiotensin II levels in HF compared with normal canines. This suggests that in HF canines, the effects of TRV120027 could result from simultaneously stimulating β-arrestin pathways at the AT1R while blocking angiotensin II–mediated G-protein signaling pathways at the same receptor. In healthy canines, no changes were observed in plasma aldosterone, the secretion of which is stimulated by AT1R activation. In contrast, TRV120027 decreased aldosterone in HF canines. Of note, Lymeropoulos et al recently reported that aldosterone secretion through AT1R was mediated by β-arrestin. This was shown in vitro in the human adrenocortical zona glomerulosa cell line H295R and in vivo in normal rats with adrenal gland–specific overexpression of β-arrestin1. Transfection of the H295R cells with a dominant negative β-arrestin1 mutant significantly reduced angiotensin-induced aldosterone production. However, we did not observe a dose-dependent increase in aldosterone secretion with TRV120027 in healthy canines and in fact saw reduced aldosterone levels at high TRV120027 doses in HF canines, suggesting that the reported effect of β-arrestin1 on aldosterone secretion does not translate into the pharmacology of TRV120027.

Hematocrit decreased slightly in both healthy and HF canines, which can be explained by decreased intravascular pressure as evidenced by the reduction in right atrial pressure; this would be expected to increase fluid movement from the interstitial to the intravascular space. There were no significant changes in circulating ANP or BNP, whereas plasma cGMP decreased. Reductions in cardiac filling pressures would be expected to reduce secretion of ANP and BNP and thus decrease plasma cGMP, their second messenger generated by guanylyl cyclase A (GC-A; also referred to as the natriuretic peptide A receptor). That this is only observed for cGMP may be due to the generally lesser variability of cGMP plasma levels as compared with ANP and BNP. In addition, TRV120027 has been shown to activate eNOS through
β-arrestin. Nitric oxide generated by eNOS induces cGMP formation through soluble guanylyl cyclase in intracellular compartments that do not increase plasma cGMP. Importantly, activation of sGC has been shown to be reciprocally affected cardiorenal function of the animals. Furthermore, the HF model used in this study was induced by tachypacing and therefore the results may differ in HF of other etiologies and in other settings. In addition, studies were performed under general anesthesia, which could have also affected cardiorenal function of the animals.

In summary, we report for the first time the integrated cardiorenal and neurohumoral actions of a novel β-arrestin–biased AT1R ligand, TRV120027. In healthy and HF canines, TRV120027 showed vasodilating actions with reductions in cardiac preload and afterload and increases in cardiac output and renal blood flow. In HF, renal function was maintained despite the reduction in renal perfusion pressure. Thus, TRV120027 may not only be an innovative pharmacological tool to help elucidate G-protein–dependent versus G-protein–independent signaling at the AT1R, but it may also be a novel therapeutic in the treatment of acute HF. A schematic illustrating the actions of TRV120027 at the AT1R is shown in Figure 4.

We conclude that TRV120027, a β-arrestin–biased AT1R ligand, has cardiac unloading actions and preserves renal function in normal canines and experimental HF. The vaso-dilating effects of TRV120027 are rapidly reversible after terminating infusion, suggesting that its effects may be titratable and rapidly controlled by changes in dose. Further studies are warranted to further define its short- and long-term actions on cardiac and renal function.

Acknowledgments

We are very grateful to Gail J. Harty, Denise M. Heublein, and Sharon M. Sandberg for technical assistance and to Kent R. Bailey, PhD, for advice on linear trend analysis.

Sources of Funding

This research was supported by Trevena, Inc, and by the Mayo Foundation.

Disclosures

Drs Boerrigter and Burnett received research support from Trevena, Inc. Drs Lark, Soergel, and Violin are employed by Trevena, Inc. Dr Whalen is a former employee of Trevena, Inc, and has ownership interest (stock).

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The angiotensin II type 1 receptor (AT1R) plays a key role in regulating cardiorenal function. Classic “unbiased” AT1R antagonists block receptor coupling to both G_{i/o} and β-arrestin–mediated signals, which desensitize G-protein signaling as well as transduce G-protein–independent signals. TRV120027 is a novel β-arrestin–biased AT1R ligand, which engages β-arrestins while blocking G-protein signaling. At the AT1R, TRV120027 can inhibit angiotensin II–mediated vasoconstriction while, through β-arrestin coupling, increase cardiomyocyte contractility. We defined for the first time the acute cardiorenal actions of TRV120027 in anesthetized healthy and heart failure (HF) canines. In healthy canines, TRV120027 decreased pulmonary capillary wedge pressure and systemic and renal vascular resistances while increasing cardiac output, renal blood flow, glomerular filtration rate, and urinary sodium excretion. In canines with HF induced by tachypacing, TRV120027 decreased mean arterial pressure, right atrial pressure and pulmonary capillary wedge pressure, and systemic and renal vascular resistances and increased cardiac output and renal blood flow. Glomerular filtration rate and urinary sodium excretion were maintained. In a separate experiment in HF, we demonstrated that the blood pressure–lowering actions of TRV120027 were rapidly reversible, consistent with the known short half-life of TRV120027 of approximately 2 minutes. We conclude that the novel β-arrestin–biased AT1R ligand TRV120027 has cardiac unloading actions while preserving renal function. With this beneficial pharmacological profile, TRV120027 represents a novel strategy for the treatment of HF, providing a rationale for further studies in human HF.
Cardiorenal Actions of TRV120027, a Novel β-Arrestin–Biased Ligand at the Angiotensin II Type I Receptor, in Healthy and Heart Failure Canines: A Novel Therapeutic Strategy for Acute Heart Failure

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Circ Heart Fail. 2011;4:770-778; originally published online August 11, 2011;
doi: 10.1161/CIRCHEARTFAILURE.111.962571

Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

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