Cardio-Renal Effects of the A1 Adenosine Receptor Antagonist SLV320 in Patients With Heart Failure

Veselin Mitrovic, MD; Petar Seferovic, MD; Slobodan Dodic, MD; Mirjana Krotin, MD; Aleksander Neskovic, MD; Kenneth Dickstein, MD; Hanka de Voogd, MD; Christiane Böcker, PhD; Dieter Ziegler, MD; Michael Godes, MD; Roumen Nakov, MD; Hans Essers, MSc; Cees Verboom, MD; Berthold Hocher, MD

**Background**—Blocking the tubuloglomerular feedback mechanism with adenosine A1 receptor antagonists seems to improve diuresis and sodium excretion without compromising the glomerular filtration rate in patients with heart failure. However, the direct cardiac effects of this compound class have not been investigated to date.

**Methods and Results**—In total, 111 patients (109 men and 2 women) received a 1-hour infusion of 5, 10, and 15 mg SLV320, an adenosine A1 receptor antagonist, placebo, or 40 mg furosemide. Mean age was 57.9 years, mean ejection fraction was 28.1%, 82 patients were of New York Heart Association class II, and 29 patients were of New York Heart Association class III. Hemodynamic parameters (heart rate, blood pressure, pulmonary capillary wedge pressure, mean pulmonary arterial pressure, systemic vascular resistance, right atrial pressure, and cardiac output) were determined. Kidney function was assessed by cystatin C measurements and by analysis of urine output and urine electrolytes. In addition, pharmacokinetics of SLV320 and ex vivo inhibition of adenosine A1 receptor activity were performed. SLV320 was well tolerated, and no serious adverse events were observed. Heart rate, blood pressure, pulmonary capillary wedge pressure, mean pulmonary arterial pressure, right atrial pressure, and cardiac output were not altered by any dose of SLV320. Pulmonary capillary wedge pressure was significantly ($P=0.04$) decreased by furosemide ($-6.2\pm5.9$ mm Hg). Systemic vascular resistance was significantly ($P=0.04$) increased in the furosemide group ($+166.70\pm261.87$ dynes $\cdot$ s $^{-1} \cdot$ cm $^{-5}$), whereas all SLV320 groups showed no significant alterations of systemic vascular resistance. Changes from baseline cystatin C plasma concentrations decreased after 10 mg SLV320 ($-0.093\pm0.137$ mg/L, $P=0.046$), whereas furosemide resulted in a significant ($P=0.03$) increase of cystatin C ($+0.052\pm0.065$ mg/L) versus baseline. All values represent mean changes±SD from baseline at 3 hours postdosing: SLV320 (10 and 15 mg) increased significantly sodium excretion and diuresis compared with placebo during the 0- to 6-hour collection period postdosing.

**Conclusions**—SLV320 infusion shows no immediate effects on cardiac hemodynamics. SLV320 might improve glomerular filtration rate while simultaneously promoting natriuresis and diuresis.

**Clinical Trial Registration**—clinicaltrials.gov Identifier: NCT00160134.

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**Key Words:** adenosine A1 antagonist ‧ renal function ‧ congestive heart failure ‧ diuretics

In the past decade, it became more and more evident that patients suffering from both chronic renal failure and chronic heart failure (HF) are characterized by a poor outcome with respect to morbidity and mortality. The underlying mechanisms are not yet completely understood. Inflammation, vascular and tissue calcification, anemia, and direct cardiotoxic effect of yet unknown molecules that accumulate in patients with impaired kidney function are suspected to be

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causal factors leading to the cardiorenal syndrome. Thus, new approaches are urgently needed. There is already evidence that risk factors related to kidney function correlate much better with outcome compared with classical cardiac risk factors in patients with HF. Moreover, a recent study

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From the Kerckhoff-Klinik, Department of Cardiology and CardioSurgery (V.M.), Bad Nauheim, Germany; Klinicki Center Serbia (P.S.), Institut za kardiovaskularne bolest, Belgrad; Medicinski Facultet Uni Novi Sad (S.D.), Institut za kardiovaskularne bolest, Sremska Kamenica; Clinical Centre Beznjiska Kosa, Department of Cardiology (M.K.), Zemun; Dedinje Cardiovascular Institute Milana Tepica 1 (A.N.), Belgrade, Serbia; Stavanger Universitetssykehus Kardiologisk divisjon (K.D.), Stavanger, Norway; Solvay Pharmaceuticals Research Laboratories (H.V., C.B., D.Z., R.N., H.E., C.V., B.H.), Hannover, Germany and Weesp, The Netherlands; Solvay Pharmaceuticals, Hans Böckler Allee 20, D-30173 Hannover, Germany. E-mail berthold.hocher@charite.de

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demonstrated that improvement of renal function by an A1 adenosine receptor antagonist in patients with acutely decompensated HF may translate in reduced hospitalization and 60-day mortality.4

The adenosine system is involved in several key functions of both the kidney and the heart. Adenosine acts through 4 different receptors: A1, A2A, A2B, and A3.5 In the kidney, adenosine plays a key role in the tubuloglomerular feedback mechanism and thus exerts an inhibitory action on glomerular hemodynamics and glomerular filtration rate (GFR) through A1 receptors.6,7 In addition, adenosine has antinatriuretic (and antidiuretic) effects, through activation of tubular A1 receptors, that promote sodium reabsorption.8–10 In the heart, A1 receptor activation may be deleterious with regard to ischemia/reperfusion injury, through promotion of neutrophil chemotraction and adhesion,11–13 whereas A2 and/or A3 activation/reperfusion injury, through promotion of neutrophil chemotraction and adhesion,11–13 whereas A2 and/or A3 activation is protective in this setting. Given these complex preclinical data, human studies addressing the cardiac effects of A1 adenosine antagonists are urgently needed.

In patients with congestive HF, A1 receptor antagonists might increase diuresis without compromising GFR, and is in contrast to the loop diuretic furosemide, which increases diuresis at the expense of a decreased GFR.14,15 Moreover, loop diuretics might be harmful for patients with acutely decompensated HF.16

SLV320, a pyrrolopyrimidine derivative, is a selective adenosine A1 receptor antagonist.17 Use of this compound in rats with 5/6 nephrectomy showed that SLV320 prevented the development of uremia-related cardiac fibrosis.17 In line with this finding are data indicating that cardiac overexpression of the A1 adenosine receptor in mice causes myocardial fibrosis associated with an increased mortality.18

In this clinical trial, we focused on short-term effects. This was a randomized, placebo-controlled, double-blind, multicenter, parallel-group, single-dose study evaluating hemodynamic and renal efficacy as well as safety of SLV320 in patients with stable HF in comparison to furosemide and placebo.

Methods

This was a randomized, placebo-controlled, double-blind, multicenter, parallel-group, single-dose study to evaluate hemodynamic and renal effects of single IV doses of the A1 adenosine receptor antagonist, SLV320 (5, 10, and 15 mg as 1-hour infusion) compared with placebo (1-hour saline infusion) or furosemide (40 mg as 5-minute bolus and 55-minute saline) during 12-hour right heart catheterization in subjects with stable HF requiring diuretic treatment. The primary end point was to evaluate the maximum reduction in the pulmonary capillary wedge pressure (PCWP) from baseline during the first 12 hours (regardless when the maximum reduction may occur during this 12 hours) after dosing with any IV dose of SLV320 in subjects with HF (New York Heart Association [NYHA] classes II–III) requiring diuretics compared with dosing with placebo.

The study enrolled patients at 6 clinical sites. Inclusion criteria included NYHA classes II–III HF with an ejection fraction of <35% measured by echocardiography at screening and the presence of edema, despite a daily furosemide dose of at least 80 mg. Baseline GFR, as measured by estimated creatinine clearance at screening (MDRD [the Modification of Diet in Renal Disease study] formula), was at least 30 mL/min per 1.73 m² or serum creatinine was <1.9 mg/dL. Main exclusion criteria were (1) the subjects' conditions were so unstable that they required hospitalization (for cardiovascular disease) or adjustment of background medications for HF; (2) subjects with a sitting systolic blood pressure of <90 mm Hg (at screening); (3) subjects with 2nd or 3rd degree atrioventricular block or sick sinus syndrome; (4) subjects with a heart rate of <50 or >110 bpm as measured on ECG (at screening); and (5) subjects with a transplanted heart. The study protocol was approved by all participating institutional review boards, and all patients gave written informed consent for participation in this study.

The clinical study was divided into 4 periods:

- Screening (visit 1 [day 7 to day 2])
- Pretreatment period (visit 2a [day 1]) (catheterization session)
- Treatment period (visit 2b [day 1]) (60-minute infusion, 12-hour postdosing hemodynamic assessments, pharmacokinetics measurements, and observation for 24 hours)
- Posttreatment period (visit 3 [day 3 to day 8]) (follow-up)

The plan was to enroll 110 subjects in the study with 22 subjects randomly assigned to each treatment group. Each subject received 1 infusion of SLV320, placebo, or furosemide. Subjects were assessed at screening as eligible and willing to participate in the clinical study. Subjects who met all of the inclusion and none of the exclusion criteria were catheterized at the pretreatment period (day 1) and received 1 of the following treatment regimens:

- SLV320 5 mg IV
- SLV320 10 mg IV
- SLV320 15 mg IV
- Placebo IV (saline)
- Furosemide 40 mg IV

The randomization was performed in blocks of 5 and stratified by center.

The doses of SLV320 were chosen based on the phase 1 data in healthy volunteers; the selected doses showed no safety signals and had clear diuretic properties. Study medication was infused over a 1-hour period. Baseline medications, such as diuretics, angiotensin-converting enzyme inhibitors, β-blockers, and nitrates and other vasodilators, if applicable, were withheld until after the 12-hour postdosing hemodynamic assessments were completed, unless required in an emergency. Baseline medications varied substantially in the patients with HF and were withheld during hemodynamic assessment to get a more uniform situation in this very first phase 2 trial with SLV320.

The investigation started in the early morning meaning that all patients took their last usual oral drug administered the evening before. At pretreatment, after a resting period of 30 minutes, cardiac output (CO) and heart rate measurements were determined at 10-minute intervals until baseline stability had been established. Baseline stability was defined as CO and heart rate measurements showing <10% variability at 2 consecutive time points.

A balloon-tipped, thermol dilution pulmonary artery catheter was inserted using standard percutaneous techniques. The antecubital, subclavian, femoral, or internal jugular venous approach was allowed. Each participating study center was to follow their standard procedures.

The hemodynamic variables were measured at: 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 hours. Postdosing is defined relative to the start time of the infusion.

The following hemodynamic measurements were made: PCWP in mm Hg; CO in L/min (determined by thermodilution); heart rate in bpm (from ECG); pulmonary arterial systolic pressure and pulmonary arterial diastolic pressure in mm Hg; systemic arterial systolic pressure and systemic arterial diastolic pressure in mm Hg; mean arterial pressure in mm Hg; and right atrial pressure (RAP) in mm Hg.

At each measurement time point for hemodynamic variables, measurements were recorded 3 times (5 times for subjects with atrial fibrillation) and the mean captured on the case report form.

Efficacy Data for Urinary Output and Excretions

Serum creatinine and cystatin C concentrations were measured immediately before dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, and 8 hours. Urine was collected during the 0 to 6- and 6 to 12-hour intervals. Patients were asked to empty their bladder before collec-
tion start and at the end of each collection period. The volume of urine and excretion of sodium, potassium, chloride, and uric acid were evaluated.

Pharmacokinetic Measurements
Blood samples of 5 mL in heparinized tubes for determining the plasma concentrations of SLV320 were collected during the treatment period at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 8, 12, and 24 hours after start of the infusion. The samples were taken from the right heart catheter line (right atrium port). SLV320 plasma concentrations were determined by validated liquid chromatography tandem mass spectrometry methods. The concentrations of SLV320 were calculated by suitable linear regression curve fitting. Quality control samples were analyzed throughout the study. The measured concentrations of these quality control samples were used to determine the intraday and interday precision and accuracy of the method.

In Vitro Inhibition of A1 Receptor Activity
The ex vivo functional A1 antagonism in plasma samples was quantified as follows: yeast cells (Saccharomyces cerevisiae) expressing the human adenosine A1 receptor were inoculated in LT-medium (SD medium lacking leucine and tryptophan, pH 6.8) to a density of 3×10⁵ cells/mL, and incubated at 30°C with agitation for 16 hours (overnight). The plasma samples were stored at −20°C and were thawed on the day of the experiment. Assays were conducted in a final volume of 100 μL in 96-well microtiter plates. Incubations were performed as follows: 10 μL plasma was added followed by 10 μL of the A1 receptor agonist 5′-N-ethylcarboxamidoadenosine (final concentration, 1 μmol/L in water) and 80 μL of the cell suspension (final cell concentration, 1.6×10⁶ cells/mL). Additional incubations without plasma (replaced by water) acted as 0% inhibition (100% stimulation) controls. Furthermore, incubations without 5′-N-ethylcarboxamidoadenosine and with water replacing plasma were included to determine basal fluorescence by β-galactosidase activity. Control plasma (without compound) had no inhibitory effect on the adenosine A1 receptor. All samples were tested in duplicate, and the means are reported. All steps were performed under sterile conditions. The plates were agitated briefly and incubated for 4 hours at 30°C. The β-galactosidase activity was determined using the fluorescent β-galactosidase substrate fluorescein-di-β-galactopyranoside (FDG, Molecular Probes). The FDG solution contains 2.5% Triton X-100 to harvest the cells. FDG/Triton X-100 was added to all wells at 20 μL/well (final concentration, 80 μmol/L). After 45 minutes of incubation, the reaction was stopped by use of 20 μL/well of a 1 mol/L Na₂CO₃. Relative fluorescence intensity was determined using a fluorometer (excitation 485 nm and emission 535 nm).

Statistical Methods
The sample size estimates were based on the primary end point—maximum change from baseline in PCWP. The sample size was calculated using ANOVA with Dunnett’s test to correct for the multiple comparisons. A sample size of 22 subjects per treatment group was considered to be sufficient to detect a 5-mm Hg difference in change from baseline in PCWP (a between-subject standard deviation of 5 mm Hg was assumed) between SLV320 and furosemide at an overall 5% significance level with a power of 80%.

All efficacy parameters were analyzed using an analysis of covariance (ANCOVA) with the baseline value as covariate and treatment, country, and NYHA classification as factors. The significance of the treatment effects of each of the 3 dose levels of SLV320 was examined in comparison with placebo and furosemide. Furosemide and placebo were also compared. P values <0.05 were considered significant. We followed the intent-to-treat principles. Data are presented with (Dunnett’s test) and without correction for multiple comparison. Only if the overall F test for treatment is significant, subsequent significant unadjusted pairwise comparisons are considered. The results are presented for the entire study population, consisting of all randomized subjects who receive the single dose of study medication and have at least 1 evaluable efficacy measurement at baseline and at postbaseline. Continuous demographic and baseline variables presented are summarized using mean, median, standard deviation, minimum, maximum, and number of available observations. Categorical demographic and baseline variables are summarized by counts and percents.

Results
One hundred fifty-one patients were screened for the study: 40 patients did not fulfill the inclusion and exclusion criteria and were thus rejected from participation. The remaining 111 patients were included in the study. This was 1 patient more than planned. The first subject’s first visit was January 5, 2005; the last subject’s last visit was November 29, 2005. Patients’ characteristics (109 men and 2 women) are given in Tables 1 and 2. Mean age was 57.9 years, mean ejection fraction was 28.10, 82 patients were of NYHA class II, and 29 patients were of NYHA class III. All patients were taking angiotensin-converting enzyme inhibitors: 86 took β-blockers, 47 took cardiac glycosides, 5 were on thiazides, and 2 were receiving spironolactone. There were no significant differences between the placebo group, the 3 SLV320 treatment groups, and the furosemide group (Tables 1 and 2), although mean baseline cystatin C was lowest in the placebo group.

Pharmacokinetics
Plasma concentrations of SLV320 after IV infusions are presented in Figure 1 in semilogarithmic scale. The plasma half-life was 1.42, 1.50, and 2.13 hours for the 5-, 10-, and 15-mg SLV320 infusions, respectively.

Adenosine Antagonism
The ability of a patient’s plasma to inhibit A1 adenosine receptor activity was similar for the SLV320 10- and 15-mg treatment groups and only slightly lower for the SLV320 5-mg treatment group.

In contrast, there were no changes from baseline adenosine A1% inhibition time profiles (Figure 2) for placebo and 40-mg furosemide treatment groups, indicating no relevant A1 adenosine receptor antagonistic activity of placebo and furosemide treatment.

Kidney Function
All 3 SLV320 treatment groups showed a small mean decrease from baseline in cystatin C during the 12-hour postdosing period, but compared with placebo, these differences did not reach statistical significance after Dunnett’s correction. In contrast, cystatin C showed an increase from baseline in the furosemide treatment group during the 12-hour postdosing period. The differences between furosemide and each of the 3 SLV320 doses were statistically significant at all measured time points after Dunnett’s correction. This was especially notable with the first 4 hours postdosing.

Because our study is more an exploratory phase 2 study, we also present data without Dunnett’s test for adjustment for multiple comparisons: urinary sodium and chloride excretion increased in a dose-dependent manner in patients receiving 5, 10, or 15 mg SLV320 IV, whereas potassium (data not shown) excretion was not affected. Urine volume was also increased in a dose-dependent manner during the first 6 hours after IV administration of SLV320. The diuretic effect of all
Table 1. Patient Population

<table>
<thead>
<tr>
<th></th>
<th>SLV320 (n=22)</th>
<th>Placebo (n=22)</th>
<th>Furosemide (n=22)</th>
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<tbody>
<tr>
<td></td>
<td>5 mg</td>
<td>10 mg</td>
<td>15 mg</td>
</tr>
<tr>
<td>Age, y</td>
<td>58.4±11.5</td>
<td>59.2±12.5</td>
<td>56.9±9.6</td>
</tr>
<tr>
<td>Male/female</td>
<td>23/0</td>
<td>21/1</td>
<td>21/1</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.79±0.1</td>
<td>1.74±0.1</td>
<td>1.75±0.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>89.7±16.6</td>
<td>78.9±14.2</td>
<td>85.3±14.3</td>
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<tr>
<td>BMI, kg/m²</td>
<td>28.0±4.5</td>
<td>26.0±3.4</td>
<td>27.8±3.4</td>
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<tr>
<td>Sitting SBP, mm Hg</td>
<td>121.9±20.1</td>
<td>127.1±15.7</td>
<td>130.2±13.9</td>
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<tr>
<td>Sitting DBP, mm Hg</td>
<td>75.9±9.7</td>
<td>79.5±9.1</td>
<td>80.5±9.9</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>90.2±11.7</td>
<td>88.9±15.2</td>
<td>98.0±14.3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>74.0±15.0</td>
<td>70.0±8.1</td>
<td>72.8±11.6</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>28.0±4.0</td>
<td>27.2±5.5</td>
<td>27.7±5.9</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>141.1±2.8</td>
<td>140.3±1.5</td>
<td>140.7±3.1</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.7±0.5</td>
<td>4.6±0.4</td>
<td>4.8±0.4</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>97±13</td>
<td>91±19</td>
<td>91±16</td>
</tr>
<tr>
<td>Cystatin C, mg/L</td>
<td>0.96±0.2</td>
<td>1.05±0.3</td>
<td>0.95±0.2</td>
</tr>
<tr>
<td>Cardiac index, L/min per m²</td>
<td>2.17±0.3</td>
<td>2.14±0.4</td>
<td>2.32±0.2</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>4.53±0.19</td>
<td>4.1±0.19</td>
<td>4.55±0.18</td>
</tr>
<tr>
<td>Pulmonary artery systolic pressure, mm Hg</td>
<td>40.9±11.6</td>
<td>42.7±13.1</td>
<td>42.1±13.1</td>
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<tr>
<td>Pulmonary artery diastolic pressure, mm Hg</td>
<td>20.3±7.2</td>
<td>19.2±6.3</td>
<td>20.3±7.7</td>
</tr>
<tr>
<td>Pulmonary artery pressure, mm Hg</td>
<td>27.8±7.7</td>
<td>29.0±7.2</td>
<td>28.9±8.0</td>
</tr>
<tr>
<td>Right atrial pressure, mm Hg</td>
<td>6.7±4.7</td>
<td>6.5±3.4</td>
<td>7.2±3.1</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dynes/s per cm⁵</td>
<td>197±113</td>
<td>200±125</td>
<td>202±133</td>
</tr>
<tr>
<td>Systemic vascular resistance, dynes/s per cm⁵</td>
<td>1126±358</td>
<td>1194±357</td>
<td>1234±305</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>17.09±4.2</td>
<td>19.36±4.2</td>
<td>18.32±4.6</td>
</tr>
</tbody>
</table>

Baseline characteristics of the 5 groups analyzed in this study. Data are given as mean±SD.

Cardiac Hemodynamics

No statistically significant differences between any SLV320 dose and placebo were observed at various time points and overall for the 12-hour postdosage assessment period for all hemodynamic outcomes after Dunnett’s correction for multiple comparison.

Treatment with placebo led to a minor nonsignificant reduction of PCWP ~2 mm Hg. Furosemide caused a significant decrease of PCWP (after Dunnett’s correction) versus placebo. The effect was, however, not significant compared with the higher doses of SLV320. The maximal effect of furosemide, ~6 mm Hg, was achieved after 3 to 5 hours.

Table 2. Heart Failure Classes and Reasons for Heart Failure in the Study Population

<table>
<thead>
<tr>
<th></th>
<th>SLV320</th>
<th>Placebo</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mg</td>
<td>10 mg</td>
<td>15 mg</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>6 (26.1)</td>
<td>3 (13.6)</td>
<td>9 (40.9)</td>
</tr>
<tr>
<td>History of hypertension, n (%)</td>
<td>6 (26.1)</td>
<td>9 (40.9)</td>
<td>14 (63.6)</td>
</tr>
<tr>
<td>NYHA classification, n (%)</td>
<td>15 (65.2)</td>
<td>16 (72.7)</td>
<td>18 (81.8)</td>
</tr>
<tr>
<td>Class II</td>
<td>17 (77.3)</td>
<td>16 (72.7)</td>
<td></td>
</tr>
<tr>
<td>Class III</td>
<td>5 (22.7)</td>
<td>6 (27.3)</td>
<td></td>
</tr>
<tr>
<td>Etiology of HF, n (%)</td>
<td>9 (39.1)</td>
<td>13 (59.1)</td>
<td>15 (68.2)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>9 (40.9)</td>
<td>7 (31.8)</td>
<td>13 (59.1)</td>
</tr>
<tr>
<td>Nonischemic heart disease</td>
<td>14 (60.9)</td>
<td>9 (40.9)</td>
<td>7 (31.8)</td>
</tr>
</tbody>
</table>

Baseline categorical characteristics of the 5 groups analyzed in the study. Data are given as n (%).
and persisted over several hours. Five milligrams of SLV320 had a placebo-like effect, whereas 10 and 15 mg of SLV320 showed a nonsignificant trend of PCWP values consistently lower than placebo (Figure 5).

RAP was not reduced by any of the SLV320 doses compared with placebo, whereas furosemide decreased RAP (Figure 5). Systemic and pulmonary vascular resistance were not altered by any of the SLV320 doses. By contrast, systemic vascular resistance was significantly \( P < 0.05 \), without Dunnett’s correction) increased in the furosemide group compared with placebo at time points 0.5, 1, 3, 4, and 5 hours postdosing. At 1.5 and 2 hours postdosing, there was a nonsignificant trend \( P < 0.1 \) for a higher systemic vascular resistance in the furosemide-treated group.

Mean arterial blood pressure was not significantly affected by either SLV320 or furosemide (Figure 5). Mean pulmonary arterial pressure was only significantly affected by furosemide (after Dunnett’s correction) treatment, and the higher doses of SLV320 showed only a trend toward reduction of mean pulmonary arterial pressure (Figure 5).

CO remained stable after treatment with SLV320, whereas furosemide showed a trend toward reduction of CO (data not shown).

Safety Results
No deaths or other serious adverse events were reported during the study. Of the total 111 subjects, 11 (9.9%) subjects had at least 1 treatment-emergent adverse event. Only 1 subject terminated the study prematurely because of a treatment-emergent adverse event (“sacral pain”), which was severe and considered unrelated to SLV320 treatment. Five (4.5%) subjects had 6 drug-related treatment-emergent adverse events. The treatment-emergent adverse events considered related to SLV320 were “dizziness,” 1 subject (5-mg SLV320 group); “nausea,” 2 subjects (5- and 10-mg SLV320 group); “transient hypertension” 1 subject (5-mg SLV320 group), and “transient hypotension,” 2 subjects (5- and 15-mg SLV320 group). For these events, a dose-response relationship could not be established.

Discussion
This study simultaneously analyzed cardiac and renal effects of an A1 adenosine receptor antagonist in patients with stable HF. SLV320 increased sodium and chloride excretion as well as diuresis in a dose-dependent manner. In contrast to furosemide treatment, SLV320 treatment reduced cystatin C plasma concentration. The hemodynamic measurements revealed no safety concerns; total peripheral resistance was not altered after SLV320 treatment, whereas furosemide treatment increased total peripheral resistance in these patients with HF.

Cardiac Effects
PCWP was not significantly lowered by any dose of SLV320 at any time point. This finding is not unexpected, given the mainly renal mode of action of an A1 adenosine receptor antagonist (Figure 5). Overall, SLV320 had also no significant effect on pulmonary artery pressure and RAP. As reported by others (for review see Ref. 16), furosemide treatment led to a significant and immediate increase in total peripheral resistance. This was not observed by any dose of SLV320. All SLV320 doses were neutral with respect to systemic vascular resistance. This is clearly an advantage for patients with HF.

Renal Effects
This study demonstrates that SLV320 increased sodium and chloride excretion as well as diuresis in a dose-dependent manner without compromising plasma cystatin C. Plasma cystatin C even decreased in the 10-mg SLV320 IV group. In contrast, furosemide treatment increased plasma levels of cystatin C, indicative of worsening of kidney function. These data with respect to renal function fit well with recent studies performed in patients with HF with other A1 receptor antagonists.14,15,19–21

A1 adenosine receptors have a dual mode of renal action7–10: (1) activation of tubular A1 adenosine receptor increases tubular...
sodium reabsorption and finally decreases sodium concentration at the macula densa; (2) activation of A1 adenosine receptor at the vasa afferentia of the glomeruli plays a key role in the tubuloglomerular feedback mechanism by exerting an inhibitory action on glomerular hemodynamics and GFR. Thus, both A1 adenosine receptor-mediated effects will have opposite effects on GFR. This might explain the bell-shaped dose response curve of all investigated A1 adenosine receptor antagonists in patients with HF with respect to control of diuresis and GFR.14,15,19–21 SLV320 had the clearest effect on reduction of GFR measured by changes of cystatin C from baseline with 10 mg SLV320, whereas the lower and higher doses were less effective.

The long-lasting effect (at least 8 hours) on cystatin C cannot be explained by the pharmacokinetics of SLV320. Control of kidney function is complex, and the biological effects seem to last much longer than the half-life of the compound. Similar observations were made with another A1 adenosine receptor antagonist in patients with HF, thus this is not a compound-related effect but rather a class effect.21

The effects on sodium excretion and diuresis are most likely mediated by means of direct tubular effects of A1 adenosine receptor antagonists.22

This study is the first to analyze the short-term GFR effects by measuring cystatin C in a clinical HF trial; however, others are also currently addressing this issue (see http://clinicaltrials.gov/ct2/show/NCT00561483?term=cystatin+C&rank=1). Cystatin C is most likely a better marker for GFR compared with creatinine especially in patients with HF because cystatin C is independent of muscle mass and less dependent on age and sex.23–25 Cystatin C was recently used as a marker for glomerular filtration in studies analyzing pathways of acute

Table 3. Urine Excretion and Sodium Excretion During the 7-to-12-h Postdosing Period

<table>
<thead>
<tr>
<th>SLV320</th>
<th>5 mg</th>
<th>10 mg</th>
<th>15 mg</th>
<th>Placebo</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine excretion (in mL/h) during the 7- to 12-h collection period</td>
<td>105.3±74.9</td>
<td>79.9±43.3</td>
<td>110.1±87.1</td>
<td>85.6±72.7</td>
<td>90.1±72.6</td>
</tr>
<tr>
<td>Sodium excretion (in mmol) during the 7- to 12-h collection period</td>
<td>57.3±25.1</td>
<td>58.2±31.0</td>
<td>78.1±58.7</td>
<td>67.8±48.6</td>
<td>52.8±37.1</td>
</tr>
</tbody>
</table>

There were no statistical significant differences between groups. Data are given as mean±SD.
renal failure, indicating that this biomarker of renal function is suitable for the detection of rapid changes of kidney function.\textsuperscript{26–32} This SLV320 study suggests that cystatin C measurements offer a new and practical method for monitoring changes in kidney function after acute IV administration of a compound that might affect GFR. Although the experience in acute renal failure and the aforementioned advantages of cystatin C measurements are compelling, systematic head-to-head comparisons of the different methods to measure short-term GFR alterations in patients with HF are needed to confirm the applicability of the new method.

Safety
There were no major safety concerns in patients with HF. A recent study performed with the A1 adenosine receptor antagonist KW-3902 in patients with HF\textsuperscript{20,21} reported seizures as side effect. Because A1 adenosine receptors play a critical role in the central nervous system,\textsuperscript{33–35} seizures might be a class effect rather than a compound-related side effect. Although there were no exclusions for patients with previous seizures and organic brain disease such as brain surgery or brain tumor in the SLV320 study, it is possible that the lack of seizures is due to the limited number of patients (n=67) exposed to the active drug. Further studies are clearly needed to assess whether SLV320 has a better safety profile compared with other A1 adenosine antagonists. With respect to safety, it is also important to note that none of the hemodynamic parameters showed any safety issue (Figure 5). By contrast, most of these parameters were influenced in a way—although not reaching statistical significance—that favors improvement in patients with HF. This is important to

Figure 4. Time course of plasma cystatin C concentrations after administration of 5, 10, and 15 mg SLV320 IV, placebo, or 40 mg furosemide IV. Samples from all 111 study patients were used for this analysis. Error bars are ±1 SEM. \(*P<0.05, \#P<0.05\), for furosemide versus placebo at the same time point; \(\#P<0.05\), for 10 mg of SLV320 versus placebo at the same time point.

Figure 5. Time course of pulmonary capillary wedge pressure, RAP, pulmonary vascular resistance, systemic vascular resistance, mean arterial pressure, and mean pulmonary arterial pressure after administration of 5, 10, and 15 mg SLV320 IV, placebo, or 40 mg furosemide IV. All 111 patients could be used for hemodynamic assessments. Error bars are ±1 SEM. \(*P<0.05\), for furosemide versus placebo at the same time point.
note because there were suspicions based on preclinical data that a blockade of the A1 receptor might be harmful at least in patients with coexisting coronary heart disease. A significant proportion of the patients included in the study suffered from ischemic heart disease. These patients had the same efficacy and safety profile as those with nonischemic heart disease (data not shown).

**Study Limitations**

We have to acknowledge the following study limitations: (1) assessment of GFR was not performed by a clearance-based technique such as inulin clearance or creatinine clearance; (2) only a limited dose range was investigated; (3) the combination of furosemide and an A1 receptor antagonist was not studied; and (4) the study was performed in patients with stable HF with a clinical need for being treated with furosemide. However, the target population for an IV formulation is more likely acutely decompensated. Because of the early phase of development, patients with stable HF were selected for initial evaluation.

**Clinical Implications**

Renal insufficiency represents an independent risk factor for disease progression and mortality in patients with HF. Moreover, it was already shown that parameters describing kidney function have a better prognostic prediction for outcome than purely cardiac biomarkers in patients with HF. The SLV320 data as well as other studies suggest that a blockade of the A1 receptor might be of special interest in several subgroups of patients with HF: (1) patients with diuretic resistance; (2) patients with acutely decompensated HF with worsening of GFR; (3) patients with chronic HF requiring long-term treatment with diuretics; and (4) patients with HF with a pronounced cardiac remodelling or fibrosis.

With respect to acutely decompensated HF, it is important that SLV320 did not alter total peripheral resistance, whereas furosemide treatment is known to increase total peripheral resistance as shown in the study. Increase of total peripheral resistance in patients with an acutely failing heart is clearly an adverse effect of loop diuretics. However, up to now, there was no alternative approach.

Our study is the first to show that diuresis can be achieved in patients with HF without compromising total peripheral resistance.

However, it is important to consider that the diuretic property of adenosine A1 receptor antagonists is much weaker compared with loop diuretics. Thus, these drugs will most likely be added to loop diuretics, especially in patients with acute HF. Additional studies are needed to analyze the cardiac hemodynamic effects in patients with HF receiving both loop diuretics and A1 adenosine receptor antagonists.

In conclusion, A1 adenosine receptor antagonism has no immediate hemodynamic effects in patients with HF. However, one dose (10 mg) of SLV320 improved kidney function (this statement is based on a positive F test followed by pairwise comparisons). Thus, we conclude that A1 adenosine receptor antagonism by SLV320 might improve kidney function compared with furosemide while simultaneously promoting natriuresis and diuresis in patients with HF. SLV320 had a favorable safety profile. Thus, SLV320 as an A1 adenosine receptor antagonist might represent a new therapeutic strategy for the treatment of patients with HF.

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**Disclosures**

None.

**References**


Heart failure (HF) is still one of the most common reasons for hospitalization in developed countries with an aging population worldwide. A substantial proportion of patients with acute HF show significant renal dysfunction. Treatment of congestive HF itself is limited by worsening renal function, despite persistent volume overload. This connection between HF and renal dysfunction has been termed the cardiorenal syndrome and has made treatment of patients with stable and unstable HF difficult. Blocking the tubuloglomerular feedback mechanism with adenosine A1 receptor antagonists such as SLV320 seems to improve diuresis and sodium excretion without compromising glomerular filtration rate in patients with HF. This study confirmed existing clinical data in patients with chronic HF, however, by use of, for the first time in a hemodynamic chronic HF clinical study, a biomarker for glomerular filtration rate: cystatin C. By using this biomarker, we demonstrated a fast and sustained improvement of GFR after SLV320 infusion. Thus, cystatin C might be a biomarker even suitable to describe acute renal effect of new drugs in clinical research. This study also analyzed, for the first time, direct cardiac effects of A1 adenosine receptor antagonists. We demonstrated that SLV320 has no direct effects on heart rate, blood pressure, pulmonary capillary wedge pressure, mean pulmonary arterial pressure, systemic vascular resistance, right atrial pressure, and cardiac output. We concluded that SLV320 infusion shows no immediate effects on cardiac hemodynamics. SLV320 might improve glomerular filtration rate while simultaneously promoting natriuresis and diuresis. It remains to be demonstrated that these immediate renal effects translate into clinical benefits on the long run.

**CLINICAL PERSPECTIVE**


Cardio-Renal Effects of the A1 Adenosine Receptor Antagonist SLV320 in Patients With Heart Failure

Veselin Mitrovic, Petar Seferovic, Slobodan Dodic, Mirjana Krotin, Aleksander Neskovic, Kenneth Dickstein, Hanka de Voogd, Christiane Böcker, Dieter Ziegler, Michael Godes, Roumen Nakov, Hans Essers, Cees Verboom and Berthold Hocher

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