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

Veselin Mitrovic M.D.¹, Petar Seferovic M.D.², Slobodan Dodic M.D.³, Mirjana Krotin M.D.⁴, Aleksander Neskovic M.D.⁵, Kenneth Dickstein M.D.⁶, Hanka de Voogd M.D.⁷, Christiane Böcker Ph.D.⁷, Dieter Ziegler, M.D.⁷, Michael Godes M.D.⁸, Roumen Nakov M.D.⁷, Hans Essers M.Sc.⁷, Cees Verboom M.D.⁷, Berthold Hocher M.D.⁷,⁸

¹Kerckhoff-Klinik, Dept of Cardiology and Cardiosurgery, Bad Nauheim, Germany
²Klinicki Center Serbia, Institut za kardiovaskularne bolest, Belgrad, Serbia
³Medicinski Facultet Uni Novi Sad, Institut za kardiovaskularne bolest, Sremska Kamenica, Serbia
⁴Clinical Centre Bezanijska Kosa, Department of Cardiology, Zemun, Serbia
⁵Dedinje Cardiovascular Institute Milana Tepica 1, Belgrade, Serbia
⁶Stavanger Universitetssykehus Kardiologisk divisjon, Stavanger, Norway
⁷Solvay Pharmaceuticals Research Laboratories, Hannover, Germany and Weesp, The Netherlands
⁸Center for Cardiovascular Research/Department of Pharmacology and Toxicology, Charité, Campus Mitte, Berlin, Germany

Corresponding author:

Prof. Dr. Berthold Hocher
Solvay Pharmaceuticals
Hans Böckler Allee 20
D-30173 Hannover, Germany
Phone:+49 511 857 2074
Fax: +49 511 857 2464
e-mail: berthold.hocher@charite.de
Abstract

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

Methods and Results: In total 111 patients (109 male/2 female) received a one-hour infusion of 5, 10, 15 mg SLV320, an adenosine A1 receptor antagonist, placebo or 40 mg furosemide, respectively. Mean age was 57.9 years, mean ejection fraction was 28.1 %, Eighty-two patients were of NYHA class II, twenty-nine patients were of NYHA class III. Hemodynamic parameters (heart rate, blood pressure, PCWP, MPAP, SVR, RAP, and CO) were determined. Kidney function was assessed by cystatin C measurements, analysis of urine output and urine electrolytes. In addition, pharmacokinetics of SLV320 and ex vivo inhibition of adenosine A1 receptor activity was performed. SLV320 was well tolerated, no serious adverse events were observed. Heart rate, blood pressure, PCWP, MPAP, RAP, and CO was not altered by any dose of SLV320. PCWP was significantly (p=0.04) decreased by furosemide (-6.2 ± 5.9 mmHg). SVR was significantly (p=0.04) increased in the furosemide group (+166.70 ± 261.87 dynes/sec*cm⁻⁵), whereas all SLV320 groups showed no significant alterations of SVR. Changes from baseline cystatin C plasma concentrations decreased after 10 mg SLV320 (-0.093 ± 0.137 mg/l, p=0.046), whereas furosemide resulted in a significantly (p=0.03) increase of (cystatin C (+0.052±0.065 mg/l) versus baseline. All values represent mean changes ± SD from baseline at 3 hours post doing:SLV320 (10 mg and 15 mg) increased significantly sodium excretion and diuresis as compared to placebo during the 0 – 6 hours collection period post dosing.

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

Key Words

adenosine a1 antagonist • renal function • congestive heart failure • diuretics

Total word count: 5987xxxxx
Introduction

In the past decade it became more and more evident that patients suffering from both chronic renal failure and chronic heart failure are characterized by a poor outcome with respect to morbidity and mortality. The underlying mechanisms are so far not completely understood. Inflammation, vascular and tissue calcification, anemia, and direct cardio-toxic effect of yet unknown molecules that accumulate in patients with impaired kidney function are suspected to be causal factors leading to the cardio-renal syndrome (1, 2). Thus new approaches are urgently needed. There is already evidence that risk factors related to kidney function correlate much better with outcome as compared to classical cardiac risk factors in HF patients (3). Moreover, a recent study demonstrated that improvement of renal function by an A1 adenosine receptor antagonist in acutely decompensated HF patients may translate in reduced hospitalization and 60 day-mortality (4).

The adenosine system is involved in several key functions of both kidney and heart. Adenosine acts via 4 different receptors: A1, A2A, A2B and A3 (5). In the kidney adenosine plays a key role in the tubuloglomerular feedback (TGF) mechanism, and thus exerts an inhibitory action on glomerular hemodynamics and glomerular filtration rate (GFR) via A1 receptors (6, 7). In addition, adenosine has anti-natriuretic (and anti-diuretic) effects, through activation of tubular A1 receptors which promote sodium re-absorption (8, 9, and 10). In the heart, A1 receptor activation may be deleterious with regard to ischemia/reperfusion injury, through promotion of neutrophil chemoattraction and adhesion (11, 12, 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 heart failure A1 receptor antagonists might increase diuresis without compromising GFR, 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 decompensate heart failure (16). SLV320, a pyrrolo-pyrimidine-derivative, is a selective adenosine A1 receptor antagonist (17). Using this compound in rats with 5/6 nephrectomy, it was shown that SV320 prevented the development of uremia related cardiac fibrosis (17). In line with this are data indicating that cardiac over-expression of the A1 adenosine receptor in mice causes myocardial fibrosis associated with an increased mortality (18).
In the current clinical trial, we focused on short-term effects. This was a randomized, placebo-controlled, double-blind, multi-center, parallel-group, single dose study, analyzing the cardiac and renal efficacy as well as safety of SLV320 in stable heart failure patients in comparison to furosemide and placebo.

**Methods**

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

The study enrolled patients at 6 clinical sites. Inclusion criteria included New York Heart Association Class II-III heart failure with an ejection fraction less than 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 formula), was at least 30 mL/min per 1.73 m² or serum creatinine was <1.9 mg/dl. Main exclusion criteria were: a) The subject's condition was so unstable that they required hospitalization (for cardiovascular disease) or adjustment of background medications for HF, b) Subjects with a sitting SBP < 90 mmHg (at screening), c) Subjects with 2nd or 3rd degree atrio-ventricular block or sick sinus syndrome, d) Subjects with a heart rate of < 50 or >110 bpm as measured on ECG (at screening), d) 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 four periods:

- Screening (Visit 1 [Day -7 to Day -2])
- Pre-Treatment Period (Visit 2a [Day -1]) (Catheterization Session)
- Treatment Period (Visit 2b [Day 1]) (60-minute infusion, 12-hour post-dosing hemodynamic assessments, PK measurements and observation for 24 hours)
Post-Treatment Period (Visit 3 [Day 3 to Day 8]) (Follow-up)
The plan was to enrol 110 subjects in the study with 22 subjects randomized to each treatment group. Each subject received one 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 pre-treatment period (day -1) and received one of the following treatment regimes:

- SLV320 5 mg i.v.
- SLV320 10 mg i.v.
- SLV320 15 mg i.v.
- placebo i.v. (saline)
- furosemide 40 mg i.v.

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

The dosages 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-h period. Baseline medications such as diuretics, ACE inhibitors, beta-blockers and nitrates and other vasodilators, if applicable were withheld until after the 12-hour post-dosing hemodynamic assessments were completed, unless required in an emergency. Baseline medications varied substantially in the heart failure patients and were withheld during hemodynamic assessment in order 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 administrating in the evening before. At pre-treatment, after a resting period of 30 minutes, 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 less than 10% variability at two consecutive time points.

A balloon-tipped, thermodilution pulmonary artery catheter was inserted using standard percutaneous techniques. The antecubital, subclavian, femoral or internal jugular venous approach were 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. Post-dosing is defined relative to the start time of the infusion.
The following hemodynamic measurements were made: Pulmonary Capillary Wedge Pressure (PCWP) in mmHg; Cardiac Output (CO) in L/min (determined by thermodilation); Heart rate in beats per minute (bpm) (from ECG); Pulmonary Arterial Systolic Pressure (PSP) and Pulmonary Arterial Diastolic Pressure (PDP) in mmHg; Systemic arterial systolic pressure (SBP) and systemic arterial diastolic pressure (DBP) in mmHg; Mean arterial pressure (MAP) in mmHg; Right Atrial Pressure (RAP) in mmHg.

At each measurement time point for hemodynamic variables, measurements were recorded three times (five 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 h) 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 prior to collection start and at the end of the 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 (pre-dose), 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 [RA] port). SLV320 plasma concentrations were determined by validated Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) 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 intra-day and inter-day 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*10^5 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 done as follows: 10 μl plasma was added followed
by 10 μl of the A1 receptor agonist 5’-N-ethylcarboxamidoadenosine (NECA, final
concentration 1 μM in water) and 80 μl of the cell suspension (final cell concentration:
1.6*10^5 cells/mL). Additional incubations without plasma (replaced by water) acted as
0% inhibition (100% stimulation) controls. Further, incubations without NECA and
with water replacing plasma were included to determine basal fluorescence by β-
galactosidase activity. Note that 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 done under sterile conditions. The plates were
agitated briefly and allowed to incubate for 4 h at 30°C. The β-galactosidase
activity was determined using the fluorescent β-galactosidase substrate fluorescein-
di-beta-D-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 μM). After 45 min of incubation the reaction was
stopped using 20 μl/well 1 M 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 endpoint – maximum change
from baseline in PCWP. The sample size was calculated using the analysis of
variance 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 mmHg difference in change from baseline in PCWP (between-subject
standard deviation of 5 mmHg 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 three dose levels
of SLV320 was examined in comparison with placebo and furosemide. Furosemide
and placebo were also compared. P values of less than 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 pair-wise
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 one evaluable efficacy measurement at baseline and at post-baseline. 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 in- and exclusion criteria and were thus rejected from participation. The remaining 111 patients entered the study. This was one patient more than planned. The first subject’s first visit was January 5th, 2005; last subject’s last visit was November 29th, 2005. Patient’s characteristics (109 male, 2 female) are given in table 1 and 2. Mean age was 57.90 years, mean ejection fraction was 28.10, eighty-two patients were of NYHA class II, twenty-nine patients were of NYHA class III. All patients were taking ACE inhibitors; eighty-six took beta-blockers, forty-seven took cardiac glycosides, five were on thiazides, and two were receiving spironolactone. There were no significant differences between the placebo group, the 3 SLV320 treatment groups and the furosemide group (table 1 and 2), although mean baseline cystatine C was lowest in the placebo group.

Pharmacokinetics
Plasma concentrations of SLV320 after i.v. infusions are presented in figure 1 in semi-logarithmic scale. The plasma half life was 1.42, 1.50 and 2.13 h for the 5, 10 and 15 mg SLV320 infusions, respectively.

Adenosine antagonism
The ability of patient’s plasma to inhibit A1 adenosine receptor activity was similar for the SLV320 10 mg 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 three SLV320 treatment groups showed a small mean decrease from baseline in cystatin C over the 12-hour post-dosing period, but compared to 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 over the 12-hour post-dosing period. The differences between furosemide and each of the three SLV320 doses were statistically significant at all measured time points.
points after Dunnett’s correction. This was especially notable with the first 4 hours post-dosing.

Since our study is a more 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 i.v., 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 i.v. administration of SLV320. The diuretic effect of all SLV320 dosages was lower as compared to 40 mg of furosemide (figure 3). The drug effects were no longer present/detectable in the second urine collection period: Urine excretion and sodium excretion during the 7-12 hours collection period were not significantly different between groups (table 3).

SLV320 treatment (10 mg group) reduced plasma concentrations of cystatin C significantly, whereas furosemide treatment had the opposite significant effect (figure 4). The 5 and 15 mg groups (with exception of the 15 mg 0.5 and 2 hours time points) showed a non-significant reduction of plasma cystatin C. The effect of SLV320 on cystatin C was present longer than the plasma level of SLV320.

**Cardiac Hemodynamics**

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

The treatment with placebo led to a minor non-significant reduction of PCWP about 2 mmHg. Furosemide caused a significant decrease of PCWP (after Dunnett’s correction) versus placebo. The effect was, however, not significant when compared to the higher dosages of SLV320. The maximal effect of furosemide, about -6 mmHg, was achieved after 3 to 5 hours and persisted over several hours. SLV320 of 5 mg had a placebo-like effect, whereas 10 and 15 mg of SLV320 showed a non-significant trend of PCWP values consistently lower than placebo (figure 5).

Right atrial pressure was not reduced by any of the SLV320 dosages as compared to placebo, whereas furosemide decreased right atrial pressure (Figure 5). Systemic and pulmonary vascular resistance were not altered by any of the SLV320 dosages. By contrast, systemic vascular resistance was significantly (p<0.05, without Dunnett’s correction) increased in the furosemide group as compared to placebo at time points 0.5, 1, 3, 4 and 5 hours post dosing. At 1.5 and 2 hours post dosing there
was a non-significant 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 by furosemide (figure 5). Mean pulmonary arterial pressure was only significantly affected by furosemide (after Dunnett’s correction) treatment, the higher dosages of SLV320 showed only a trend towards reduction of mean pulmonary arterial pressure (figure 5).
Cardiac output remained stable after treatment with SLV320, whereas furosemide showed a trend towards reduction of cardiac output (data not shown).

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

This study analyzed simultaneously cardiac and renal effects of an A1 adenosine receptor antagonist in stable heart failure patients. 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, whereas furosemide treatment increased total peripheral resistance in these heart failure patients.

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 PAP and RAP. As reported by others (for review see 16), furosemide treatment led to significant and immediate increase in total peripheral resistance. This was not observed by any dose of SLV320. All SLV320 dosages were neutral with respect to systemic vascular resistance. This is clearly an advantage for patients with heart failure.

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 i.v. 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 done in heart failure patients with other A1 receptor antagonists (14, 15, 19, 20, 21).

A1 adenosine receptors have a dual mode of renal action (7,8,9,10): i) activation of tubular A1 adenosine receptor increases tubular sodium reabsorption and finally decreases sodium concentration at the macula densa, ii) activation of A1 adenosine receptor at the vasa afferentia of the glomeruli plays a key role in the tubuloglomerular feedback (TGF) mechanism by exerting an inhibitory action on glomerular hemodynamics and glomerular filtration rate (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 heart failure patients with respect to control of diuresis and GFR (14, 15, 19, 20, 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 dosages were less effective.

The long-lasting effect (at least 8 h) 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 heart failure patients, 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 via 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 heart failure trial, however others are currently addressing this issue as well (see: http://clinicaltrials.gov/ct2/show/NCT00561483?term=cystatin+c&rank=1). Cystatin C is most likely a better marker for GFR as compared to creatinine especially in heart failure patients, since cystatin C is independent of muscle mass and less dependent on age and gender (23, 24, 25). Cystatin C was recently used as marker for glomerular filtration in studies analyzing pathways of acute renal failure indicating that this biomarker of renal function is suitable for the detection of rapid changes of kidney function (26-32). This SLV320 study suggests that cystatin C measurements offer a new and practical method for the monitoring of changes in kidney function after acute i.v. administration of a compound that might affect GFR. Although the experience in acute renal failure and the above mentioned advantages of cystatin C measurements are compelling, systematic head-to-head comparisons of the different methods to measure short-term GFR alterations in heart failure patients are needed to confirm the applicability of the new method."

Safety
There were no major safety concerns in patients with heart failure. A recent study done with the A1 adenosine receptor antagonist KW-3902 in heart failure patients (20, 21) reported seizures as side-effect. Since A1 adenosine receptors play a critical role in the central nervous system (33,34,35), seizures might be a class- rather a compound-related side-effect. Although there were no exclusions for patients with
prior 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 the limited number of patients (n=67) exposed to the active drug. Further studies are clearly needed to assess whether or not SLV320 has a better safety profile as compared to 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 way – although not reaching statistical significance - that favors improvement of heart failure patients. This is important to note, since there were suspicions based on preclinical data that a blockade of the A1 receptor might be harmful at least in patients with co-existing coronary heart disease (36). A significant proportion of the included patients suffered from ischemic heart disease. These patients had the same efficacy and safety profile as those with non-ischemic heart disease (data not shown).

Study limitations
We have to acknowledge the following study limitations: i) assessment of glomerular filtration rate was not performed by a clearance-based technique such as inulin clearance or creatinine clearance, ii) only a limited dose range was investigated, iii) the combination of furosemide and an A1 receptor antagonist was not studied, and iv) the study was performed in stable heart failure patients with a clinical need for being treated with furosemide. However, the target population for an i.v. formulation is more likely acutely decompensated. Because of the early phase of development, stable heart failure patients were selected for initial evaluation.

Clinical implications
Renal insufficiency represents an independent risk factor for disease progression and mortality in patient with heart failure. Moreover, it was already shown that parameters describing kidney function have a better prognostic prediction for outcome than purely cardiac biomarkers in heart failure patients (3). The SLV320 data as well as other studies (14, 15, 19, 20, 21, 22) suggest that a blockade of the A1 receptor might be of special interest in several subgroups of heart failure patients: i) patients with diuretic resistance, ii) acutely decompensated heart failure patients with worsening of GFR, iii) chronic heart failure patents requiring long-term treatment with diuretics, and iv) heart failure patient with a pronounced cardiac remodelling/fibrosis (17, 18).
With respect to acutely decompensated heart failure, it is important that SLV320 did not alter total peripheral resistance, whereas furosemide treatment is known to increase total peripheral resistance (37,38) 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 heart failure patients without compromising total peripheral resistance. However, it is important to consider that the diuretic property of adenosine A1 receptor antagonists is much weaker as compared to loop diuretics. Thus, these drugs will most likely be added to loop diuretics especially in acute heart failure patients. Additional studies are needed to analyze the cardiac hemodynamic effects in heart failure patients receiving both loop diuretics and A1 adenosine receptor antagonists.

In conclusion, A1 adenosine receptor antagonism has no immediate hemodynamic effects in heart failure patients. However, one dose (10 mg) of SLV320 improved kidney function (this statement is based on a positive F-test followed by pair-wise comparisons). We thus conclude that A1 adenosine receptor antagonism by SLV320 might improve kidney function compared to furosemide while simultaneously promoting natriuresis and diuresis in heart failure patients. 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 heart failure.

**Funding Sources**

This study was partially supported by the Deutsche Forschungsgemeinschaft (DFG) to Dr. B. Hocher.

**Conflict of interest**

Hanka de Voogd, Christiane Böcker, Dieter Ziegler, Roumen Nakov, Hans Essers Cees Verboom and Berthold Hocher are research employees of Solvay Pharmaceuticals.
Figure Legends

Figure 1: Geometric mean plasma concentration-time profiles following single 1-h infusions of 3 doses of SLV320. (Semi-Logarithmic scale) The sample sizes for pharmacokinetics are different from the numbers randomized, because one small study center was not able to perform pharmacokinetics. The 12-hour points are missing for the 10 mg and 5 mg doses of SV320, because these values were below the limit of quantification (LOQ) of the SLV320 assay.

Figure 2: Arithmetic mean change from baseline adenosine A1 receptor % inhibition-time profiles in the different treatment groups. Samples from all 111 study patients were used for this analysis.

Figure 3: Sodium excretion, chloride excretion and diuresis during the first 6 h after treatment with three different dosages of SLV320, furosemide or placebo. Samples from all 111 study patients were used for this analysis. Bars represent means +/- SEM. *: p<0.05 versus placebo.

Figure 4: Time course of plasma cystatin C concentrations after administration of 5, 10, 15 mg SLV320 i.v., placebo or 40 mg furosemide i.v. Samples from all 111 study patients were used for this analysis. Error bars are +/- 1 SEM
*: 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, right atrial pressure, pulmonary vascular resistance, systemic vascular resistance, mean arterial pressure, and mean pulmonary arterial pressure after administration of 5, 10, 15 mg SLV320 i.v., placebo or 40 mg furosemide i.v. 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;
References:


Table 1: Patient Population

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

<table>
<thead>
<tr>
<th></th>
<th>SLV320 5mg</th>
<th>SLV320 10mg</th>
<th>SLV320 15mg</th>
<th>Placebo</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>23</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.4 ±11.5</td>
<td>59.2 ±12.5</td>
<td>56.9 ±9.6</td>
<td>57.2 ±10.4</td>
<td>57.9 ±10.9</td>
</tr>
<tr>
<td>Male/Female</td>
<td>23/0</td>
<td>21/1</td>
<td>21/1</td>
<td>22/0</td>
<td>22/0</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>
<td>1.80 ±0.1</td>
<td>1.79 ±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>
<td>90.8 ±14.5</td>
<td>86.5 ±15.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ±4.5</td>
<td>26.0 ±3.4</td>
<td>27.8 ±3.4</td>
<td>27.9 ±3.5</td>
<td>27.1 ±3.5</td>
</tr>
<tr>
<td>Sitting SBP (mmHg)</td>
<td>121.9 ±20.1</td>
<td>127.1 ±15.7</td>
<td>130.2 ±13.9</td>
<td>129.0 ±15.1</td>
<td>129.6±19.8</td>
</tr>
<tr>
<td>Sitting DBP (mmHg)</td>
<td>75.9 ±9.7</td>
<td>79.5 ±9.1</td>
<td>80.5 ±9.9</td>
<td>82.8 ±8.7</td>
<td>77.7 ±10.2</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>90.2 ±11.7</td>
<td>88.9 ±15.2</td>
<td>98.0 ±14.3</td>
<td>95.8 ±16.8</td>
<td>92.0 ±11.4</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>
<td>72.5 ±12.2</td>
<td>69.5 ±10.8</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>28.0 ±4.0</td>
<td>27.2 ±5.5</td>
<td>27.7 ±5.9</td>
<td>28.5 ±5.0</td>
<td>29.2 ±4.6</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>141.1 ±2.8</td>
<td>140.9 ±1.5</td>
<td>140.7 ±3.1</td>
<td>141.7 ±3.4</td>
<td>141.2 ±2.9</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>
<td>4.6 ±0.4</td>
<td>4.7 ±0.5</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>97 ±13</td>
<td>91 ±19</td>
<td>91 ±16</td>
<td>96 ±14</td>
<td>91 ±17</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>
<td>0.81 ±0.1</td>
<td>1.03 ±0.2</td>
</tr>
<tr>
<td>Cardiac Index (L/min/m²)</td>
<td>2.17 ±0.3</td>
<td>2.14 ±0.4</td>
<td>2.32 ±0.2</td>
<td>2.17 ±0.3</td>
<td>2.24 ±0.3</td>
</tr>
<tr>
<td>Cardiac Output (L/min)</td>
<td>4.53 ±0.19</td>
<td>4.10 ±0.19</td>
<td>4.55 ±0.18</td>
<td>4.63 ±0.17</td>
<td>4.71 ±0.15</td>
</tr>
<tr>
<td>Pulmonary Artery Systolic Pressure (mmHg)</td>
<td>40.9 ±11.6</td>
<td>42.7 ±13.1</td>
<td>42.1 ±13.1</td>
<td>41.2 ±13.2</td>
<td>44.5 ±11.0</td>
</tr>
<tr>
<td>Pulmonary Artery Diastolic Pressure (mmHg)</td>
<td>20.3 ±7.2</td>
<td>19.2 ±6.3</td>
<td>20.3 ±7.7</td>
<td>18.1 ±5.6</td>
<td>20.4 ±6.7</td>
</tr>
<tr>
<td>Pulmonary Artery Pressure (mmHg)</td>
<td>27.8 ±7.7</td>
<td>29.0 ±7.2</td>
<td>28.9 ±8.0</td>
<td>27.3 ±6.5</td>
<td>29.5 ±6.9</td>
</tr>
<tr>
<td>Right Atrial Pressure (mmHg)</td>
<td>6.7 ±4.7</td>
<td>6.5 ±3.4</td>
<td>7.2 ±3.1</td>
<td>7.3 ±3.4</td>
<td>8.1 ±4.7</td>
</tr>
<tr>
<td><strong>Pulmonary Vascular Resistance</strong> (dynes/sec x cm⁻²)</td>
<td>197 ±113</td>
<td>200 ±125</td>
<td>202 ±133</td>
<td>155 ±72</td>
<td>183 ±88</td>
</tr>
<tr>
<td><strong>Systemic Vascular Resistance</strong> (dynes/sec x cm⁻²)</td>
<td>1126 ±358</td>
<td>1194 ±357</td>
<td>1234 ±305</td>
<td>1249 ±364</td>
<td>1055 ±201</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>17.09 ±4.2</td>
<td>19.36 ±4.2</td>
<td>18.32 ±4.6</td>
<td>18.73 ±6.2</td>
<td>19.18 ±5.3</td>
</tr>
</tbody>
</table>
Table 2: Heart failure classes and reasons for heart failure in the study population
Baseline categorical characteristics of the 5 groups analyzed in the study. Data are given as n (%).

<table>
<thead>
<tr>
<th></th>
<th>SLV320</th>
<th>Placebo</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5mg</td>
<td>10mg</td>
<td>15mg</td>
</tr>
<tr>
<td>Diabetes (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLV320 5mg</td>
<td>6 (26.1%)</td>
<td>3 (13.6%)</td>
<td>9 (40.9%)</td>
</tr>
<tr>
<td>SLV320 10mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLV320 15mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>7 (31.8%)</td>
<td>7 (31.8%)</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>7 (31.8%)</td>
<td></td>
<td></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>Class II</td>
<td>15 (65.2)</td>
<td>16 (72.7)</td>
<td>18 (81.8)</td>
</tr>
<tr>
<td>Class III</td>
<td>8 (34.8)</td>
<td>6 (27.3)</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td>Etiology of HF (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>9 (39.1)</td>
<td>13 (59.1)</td>
<td>15 (68.2)</td>
</tr>
<tr>
<td>Non-ischemic heart disease</td>
<td>14 (60.9)</td>
<td>9 (40.9)</td>
<td>7 (31.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14 (63.6)</td>
</tr>
</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th></th>
<th>5mg</th>
<th>10mg</th>
<th>15mg</th>
<th>Placebo</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine excretion in ml/h during the 7-12 hours collection period</td>
<td>105.3 ± 74.9</td>
<td>79.9 ± 43.3</td>
<td>110.1 ± 87.1</td>
<td>85.61 ± 72.7</td>
<td>90.1 ± 72.6</td>
</tr>
<tr>
<td>Sodium excretion in mmol during the 7-12 hours 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>

Urine excretion and sodium excretion during the 7-12 hours post dosing period. There were no statistically significant differences between groups. Data are given as mean ± SD.
Figures

Figure 1:

Mean SLV320 Plasma Concentration (ng/mL)

- 5 mg SLV320 (n = 23)
- 10 mg SLV320 (n = 20)
- 15 mg SLV320 (n = 18)

Time (hr)

- 0 6 12 18 24
Figure 2:
Figure 3:
Cystatin C change from baseline

Time (h)

Figure 4
Mean Change in Pulmonary Capillary Wedge Pressure

Mean Change in Right Atrial Pressure

Mean Change in Pulmonary Vascular Resistance

Mean Change in Systemic Vascular Resistance

Mean Change in Mean Arterial Pressure

Mean Change in Mean Pulmonary Artery Pressure

Figure 5
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 Devoogd, Christiane Böcker, Dieter Ziegler, Michael Godes, Roumen Nakov, Hans Essers, Cees N. Verboom and Berthold Hocher

_Circ Heart Fail._ published online September 24, 2009;
_Circulation: Heart Failure_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/early/2009/09/24/CIRCHEARTFAILURE.108.798389

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Heart Failure_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Heart Failure_ is online at:
http://circheartfailure.ahajournals.org//subscriptions/