Chronic Therapy With a Partial Adenosine A1-Receptor Agonist Improves Left Ventricular Function and Remodeling in Dogs With Advanced Heart Failure

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Background—Adenosine elicits cardioprotection through A1-receptor activation. Therapy with adenosine A1-receptor agonists, however, is limited by undesirable actions of full agonism, such as bradycardia. This study examined the effects of capadenoson (CAP), a partial adenosine A1-receptor agonist, on left ventricular (LV) function and remodeling in dogs with heart failure.

Methods and Results—Twelve dogs with microembolization-induced heart failure were randomized to 12 weeks oral therapy with CAP (7.5 mg BID; n=6) or to no therapy (control; n=6). LV end-diastolic and end-systolic volumes, ejection fraction, plasma norepinephrine, and n-terminal pro–brain natriuretic peptide were measured before (pre) and 1 and 12 weeks after therapy (post). LV tissue obtained at post was used to assess volume fraction of interstitial fibrosis, sarcoplasmic reticulum calcium ATPase-2a activity, expression of mitochondria uncoupling proteins (UCP) and glucose transporters (GLUT). In controls, end-diastolic and end-systolic volumes increased and ejection fraction decreased significantly from pre to post (ejection fraction, 30±2 versus 27±1%; P<0.05). In CAP-treated dogs, end-diastolic volume was unchanged; ejection fraction increased significantly after 1 week (36±2 versus 27±2%; P<0.05) with a further increase at post (39±2%; P<0.05), whereas end-systolic volume decreased. CAP significantly decreased volume fraction of interstitial fibrosis, normalized sarcoplasmic reticulum calcium ATPase-2a activity and expression of UCP-2 and UCP-3, and GLUT-1 and GLUT-2 and significantly decreased plasma norepinephrine and n-terminal pro–brain natriuretic peptide.


Key Words: adenosine receptors • heart failure • protein expression • ventricular remodeling

Adenosine is a purine nucleoside that exerts a variety of physiological actions by binding to adenosine cell surface receptor subtypes, namely A1, A2a, A2b, and A3. The cardioprotective effects of adenosine have been extensively studied and are primarily mediated by activation of A1-receptor (AIR) subtype.1,2 Activation of the AIR using full agonists, while offering potential therapeutic benefits, is limited by undesirable side effects that include bradycardia, atrioventricular blocks, and sedation and antidiuretic effects. To overcome the side effects of full AIR agonism, efforts were recently placed on tailoring compounds only to the desired pharmacological efficacy by developing partial adenosine AIR agonists that are likely to elicit beneficial therapeutic effects without giving rise to undesirable side effects.3 One such compound is capadenoson (CAP; BAY 68-4986), a partial adenosine AIR agonist.4 It has high affinity for the AIR and high selectivity over other adenosine receptors with a favorable pharmacokinetic profile evidenced by long half-life and high bioavailability.5 Previous studies with selective AIR agonists, such as tecadenoson6,7 and selodenoson,8 showed significant reduction in heart rate up to the effect of causing third-degree atrioventricular block. However, CAP has minimal effect on heart rate.5 Although CAP was previously studied in patients with stable angina,9 no studies have emerged to date that describe the effects of CAP or any other partial AIR agonist in heart failure (HF). Despite major advances during the past 2 decades that led to the development of effective therapeutic modalities for the treatment of HF, the incidence of mortality and morbidity associated with this disease syndrome remains unacceptably high necessitating continued aggressive efforts aimed at developing novel pharmaceuticals and devices to fill an unmet need. The objective of this study was to investigate, for the first time, the effects of selective partial AIR agonism with CAP on left ventricular (LV) function and remodeling in dogs with experimentally induced chronic HF.

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Methods

The canine model of intracoronary microembolization-induced chronic HF used in this study was previously described in detail.10 In this study, 12 healthy mongrel dogs, weighing between 19.4 and 24.2 kg, underwent serial coronary microembolizations, 1 to 2 weeks apart, to produce HF. Embolizations were discontinued when LV ejection fraction (EF), determined angiographically, was ≤30%. Two weeks after the target EF was reached, dogs were randomized to 12 weeks of monotherapy with CAP (7.5 mg BID; n=6) or no therapy at all (control; n=6). All the procedures were performed during cardiac catheterization under general anesthesia and sterile conditions. Induction of anesthesia was initiated with intravenous hydromorphone (0.22 mg/kg) and diazepam (0.17 mg/kg) and plane of anesthesia was maintained with 1% to 2% isoflurane. The study was approved by Henry Ford Health System Institutional Animal Care and Use Committee and conformed to the National Institute of Health Guide and Care for Use of Laboratory Animals (National Institutes of Health publication No. 85-23).

Hemodynamic, Ventriculographic, and Electrocardiographic Measurements

Measurements were made at pretreatment (before initiating therapy) and were repeated at 1 and 12 weeks after initiating therapy. Aortic and LV pressures were measured with catheter-tip micromanometers (Millar Instruments, Houston, TX). LV pressure waveforms were used to calculate LV end-systolic pressure (LVESP) and to derive peak LV +dP/dt and the time constant of isovolumic relaxation, τ. Left ventriculograms were obtained with the dog placed on its right side and recorded on 35-mm cine film at 30 frame/second during the injection of 20 mL of contrast material (ISOVU-300; Bracco Diagnostics, Inc, Princeton, NJ). Correction for image magnification was made with a radiopaque calibrated grid placed at the level of the LV. LV end-systolic volume (ESV), end-diastolic volume (EDV) and LVEF were calculated using the area-length method.11 Stroke volume (SV) was calculated as the difference between EDV and ESV. Cardiac output (CO) was calculated as the product of SV and heart rate (HR). Systemic vascular resistance (SVR) was calculated as previously described.11

In 6 of 12 dogs (3 controls and 3 CAP treated), LV pressure–volume (P–V) loops were measured using a Millar Instruments MPVS Ultra system pressure-conductance catheter (Houston, TX). Measurements were only made at pretreatment and at 12 weeks after initiating therapy. P–V loops were generated during a transient balloon occlusion of the inferior Vena Cava were used to assess the slope of the ESP volume relation (ESPVR), an index of load-independent contractility. All dogs underwent a pretreatment and a posttreatment 24-hour ambulatory ECG Holter monitoring study. Full Holter disclosures were used to measure maximum, minimum, and average HR and to evaluate rhythm abnormalities, if any. Venous venous blood was used to measure serum electrolytes and plasma levels of n-terminal pro–brain natriuretic peptide and norepinephrine by enzyme-linked immuno sorbent assay.

Echocardiographic and Doppler Flow Measurements

Echocardiographic and Doppler studies were performed using a General Electric Vivid-7 ultrasound system with a 3.5-MHz transducer and recorded on digital media for off-line analysis. LV end-diastolic circumferential wall stress was calculated as previously described.15 Trans-mitral inflow velocity waveforms, measured using pulsed-wave Doppler echocardiography, were used to calculate the time–velocity integral of mitral inflow velocity waveform representing early filling (Ei), the time–velocity integral representing left atrial contraction (A), the ratio Ei/Ai, and deceleration time (DT) of early mitral inflow velocity as previously described.13

Histomorphometric Measurements

After completion of final hemodynamic study, and while the dog was under general anesthesia, the chest was opened and the heart rapidly removed and LV tissue prepared for histological and biochemical evaluation. From each heart, 3 transverse slices (≈5 mm thick) 1 each from basal, middle, and apical thirds of the LV, were obtained. From each slice, transmural tissue blocks were obtained and embedded in paraffin blocks. Tissue blocks were also obtained from the LV free wall, mounted on cork using Tissue-Tek embedding medium, and rapidly frozen in isopentane pre-cooled in liquid nitrogen and stored at −70°C until used. The volume fraction of replacement fibrosis, volume fraction of interstitial fibrosis, myocyte cross-sectional area, a measure of cardiomyocyte hypertrophy, capillary density (CD), and oxygen diffusion distance (ODD) were measured as previously described.14,15 LV tissue from 6 normal (NL) dogs was processed in an identical manner as above and the results used for comparisons.

Biochemical Measurements

To examine the effects of therapy with CAP on myocardial energetics, protein levels of the glucose transporters, GLUT-1 and GLUT-4; mitochondria uncoupling proteins, UCP-2 and UCP-3; citrate synthase (CS); and muscle carnitine palmitoyl transferase(mCPT-1) were measured in LV tissue as was the expression of calsequestrin, a sarcoplasmic reticulum protein that does not change in HF (internal control) and porin, a mitochondrial protein that is unchanged in HF (internal control). Protein levels were measured in SDS-extract using Western blotting coupled with chemiluminescent method. Band intensity on the gels was quantified in densitometric units (du). To examine the effects of CAP on sarcoplasmic reticulum calcium cycling, thapsigargin-sensitive calcium-ATPase (SERCA-2a) activity and affinity (Ka) was determined in LV tissue homogenate as was protein level. LV tissue from 6 NL dogs was used for comparison.

Statistical Analysis

Within group comparisons of hemodynamic, ventriculographic, echocardiographic, and Doppler measures were made using repeated measures ANOVA with alpha set at 0.05. If significance was attained, comparisons between measurements made at pretreatment and those made at 1 and 12 weeks were tested using the Student–Neuman–Keuls test with P≤0.05 considered significant. A t test for 2 means was used to compare all means between the control and treated group at pretreatment, 1 week, and 12 weeks. For these comparisons, a P value of <0.05 was considered significant. To assess treatment effect, the change (Δ) in each measure from pre to post treatment (12 weeks) was calculated for each of the 2 study arms. To determine whether significant differences in Δ were present between the control group and the CAP treatment groups, a r-statistic for 2 means was used with P≤0.05 considered significant. Histomorphometric and biochemical measures among NL, control, and CAP-treated dogs were compared using 1-way ANOVA with alpha set at 0.05. If significance was attained by ANOVA, pairwise comparisons were performed using the Student–Neuman–Kuels test. For all pairwise comparisons, a P value ≤0.05 was considered significant. All data are reported as mean±SEM.

Results

None of the study dogs developed acute decompensation or died during the study and none developed ventricular or atrial arrhythmias, bradycardia, atrioventricular block, sedation, or renal abnormalities. Compared with pretreatment, there were no differences in serum creatinine or blood urea nitrogen during the course of the study in any of the study groups (Table 1).

Within Group Changes of Hemodynamic, Ventrivulographic, and Echocardiographic Measures

Hemodynamic, ventriculographic, and echocardiographic results obtained at pretreatment and at 1 and 12 weeks
post-treatment in untreated HF controls and CAP-treated HF dogs are shown in Table 1. There were no significant differences between the 2 study groups in any of the measures obtained at pretreatment. In untreated controls, HR, mean aortic pressure LV end-diastolic pressure, peak +dP/dt, LVESP, τ, SV, CO, SVR, Ei/Ai, DT, end-diastolic circumferential wall stress, and slope of the ESPVR did not change significantly during the course of 12 weeks of follow-up compared with pretreatment values (Table 1). In CAP-treated dogs, HR, mean aortic pressure, peak +dP/dt, LVESP, τ, and slope of the ESPVR were also not significantly changed during the 12-week course of therapy. In this group, LV end-diastolic pressure and end-diastolic circumferential wall stress tended to decrease and Ei/Ai and DT tended to increase but none reached statistical significance compared with pretreatment values (Table 1). Therapy with CAP significantly increased SV and CO and decreased SVR. In untreated control dogs, LVEDV was unchanged, LVESV tended to decrease and LVEF increased significantly. Improvement in EF, SV, and CO occurred as early as 1 week after initiating CAP therapy. Treatment with CAP significantly decreased plasma norepinephrine at all study time points compared with pretreatment (Table 1).

Between Groups Changes of Hemodynamic, Ventriculographic, and Echocardiographic Measures (Treatment Effect)

Comparisons between controls and CAP-treated dogs at 1 and 12 weeks are shown in Table 1. Between-group comparisons of the change (Δ) between pretreatment and posttreatment measurements are shown in Table 2. Compared with control, long-term therapy with CAP had no effect on HR, mean aortic pressure, LVESP, or slope of the ESPVR but tended to decrease LV end-diastolic pressure, τ, and EDV and tended to increase peak +dP/dt. Treatment with CAP significantly decreased ESV and SVR and increased EF, SV, and CO. Measures of LV diastolic function, however,

### Table 1. Hemodynamic, Ventriculographic, Echocardiographic-Doppler and Electrolyte Measurements Obtained at Pretreatment and at 1 and 12 Weeks After Initiating Follow-up in Control Group and Capadenoson-Treated Group

| Measures (Treatment Effect) | Pretreatment | 1 Week | 12 Weeks | 1. Between-group comparisons of the change (Δ) between pretreatment and posttreatment measurements are shown in Table 2. Compared with control, long-term therapy with CAP had no effect on HR, mean aortic pressure, LVESP, or slope of the ESPVR but tended to decrease LV end-diastolic pressure, τ, and EDV and tended to increase peak +dP/dt. Treatment with CAP significantly decreased ESV and SVR and increased EF, SV, and CO. Measures of LV diastolic function, however, |
|-----------------------------|--------------|---------|-----------|
|                             | Control      | CAP     | P Value   | Control | CAP     | P Value   | Control | CAP     | P Value   |
|                             | Value        | Value   |           | Value   | Value   |           | Value   | Value   |           |
| HR, beats per min           | 87±2.9       | 89±4.4  | 0.71      | 88±2.7  | 80±2.4  | 0.05      | 85±3.9  | 84±2.9  | 0.84      |
| mAOP, mm Hg                 | 72±2.0       | 78±2.0  | 0.06      | 81±3.9  | 84±6.5  | 0.70      | 78±1.9  | 77±5.0  | 0.86      |
| LVEDP, mm Hg                | 17±1.2       | 17±1.5  | 1.0       | 19±1.6  | 15±0.9  | 0.05      | 17±1.3  | 14±1.4  | 0.15      |
| LVEDV, mL                   | 73±4         | 70±4.2  | 0.62      | 74±3.8  | 70±4.4  | 0.51      | 83±3.1  | 72±6.6  | 0.16      |
| LVESV, mL                   | 51±3.5       | 51±3.9  | 1.0       | 52±3.4  | 45±4.1  | 0.22      | 60±2.9† | 44±6.2  | 0.04      |
| LVEF, %                     | 30±1.9       | 27±1.8  | 0.28      | 30±2.0  | 36±2.1* | 0.06      | 27±1.4† | 39±2.4* | 0.002     |
| LVESP, mm Hg                | 74±2.1       | 78±2.8  | 0.28      | 77±2.6  | 86±7.7  | 0.29      | 78±3.5  | 79±5.6  | 0.88      |
| LV +dP/dt, mm Hg/s          | 1352±126     | 1589±185| 0.32      | 1307±37| 1812±180| 0.02      | 1357±73| 1752±281| 0.20      |
| τ, ms                       | 59±6         | 60±10   | 0.93      | 73±10   | 51±4    | 0.07      | 67±13   | 48±5    | 0.21      |
| SV, mL                      | 21±2         | 19±1    | 0.29      | 22±2    | 25±1*   | 0.16      | 23±1    | 28±1*   | 0.008     |
| CO, L/min                   | 1.83±0.11    | 1.69±0.12| 0.41   | 1.91±0.14| 1.99±0.10* | 0.65     | 1.93±0.11| 2.30±0.10* | 0.03     |
| SVR, dynes/(s-cm³)          | 3292±281     | 3770±323| 0.28      | 3395±145| 3422±319| 0.94      | 3486±279| 2661±114*| 0.02      |
| Ei/Ai                       | 3.37±0.41    | 3.82±0.49| 0.50   | 3.66±0.49| 4.23±0.45 | 0.41     | 3.82±0.3 | 4.55±0.56 | 0.28      |
| DT, ms                      | 92±6         | 103±9   | 0.33      | 84±5    | 108±8   | 0.03      | 88±5    | 107±9   | 0.09      |
| LV EDWS, g/cm²              | 86±15        | 73±9    | 0.47      | 87±11   | 63±7    | 0.09      | 99±18   | 64±14   | 0.16      |
| Slope-ESPVR, mm Hg/mL (n=3 per group) | 2.72±0.62 | 1.54±0.20| 0.1   | ...     | ...     | ...       | 1.51±0.29| 1.31±0.18 | 0.57     |
| Creatinine, mg/dL           | 0.92±0.04    | 0.93±0.05| 0.88   | 0.93±0.03| 0.92±0.05 | 0.87     | 0.90±0.06| 1.00±0.07| 0.30      |
| BUN, mg/dL                  | 13±1         | 13±1    | 1.0       | 15±2    | 14±1    | 0.66      | 15±1    | 14±2    | 0.66      |
| Plasma NE, pg/mL            | ...          | 906±127 | ...     | ...     | 520±35*  | ...       | ...     | 210±21*  | ...       |

Data are shown as mean±SEM. Ai, time-velocity integral representing left atrial contraction; BUN, blood urea nitrogen; CO, cardiac output; +dP/dt indicates peak rate of change of pressure during isovolumic period; DT, deceleration time of early mitral inflow velocity; EDP, end-diastolic pressure; EDV, end-diastolic volume; EDWS, LV end-diastolic circumferential wall stress; EF, ejection fraction; Ei, time-velocity integral of the mitral inflow velocity waveform representing early filling; Ei/Ai, ratio of Ei to Ai; ESP, end-systolic pressure; ESPVR, end-systolic pressure-volume relationship; ESV, end-systolic volume; HR, heart rate; LV, left ventricular; mAOP, mean aortic pressure; NE, norepinephrine; P value, probability value of control vs capadenoson (CAP) at pretreatment, 1 wk, and 12 wk; SV, stroke volume; SVR, systemic vascular resistance; and τ, time constant of isovolumic relaxation.

*P<0.05 vs pretreatment in capadenoson group.
†P<0.05 vs pretreatment in control group.
were not significantly altered by long-term treatment with CAP as evidenced by little or no changes in Ei/Ai, DT, and end-diastolic circumferential wall stress. Compared with control, CAP therapy also significantly decreased circulating plasma levels of n-terminal pro–brain natriuretic peptide (Figure 1). There were no significant changes between groups with respect to maximum, average, and minimum HR derived from ambulatory ECG Holter monitoring studies (Table 2). Significant treatment effects were also present with respect to EF, CO, and SV as early as 1 week after initiating therapy with CAP.

**Histomorphometric Findings**

Table 3 shows histomorphometric findings. Compared with NL dogs, control HF dogs showed a significant increase in volume fraction of replacement fibrosis, volume fraction of interstitial fibrosis, ODD, and myocyte cross-sectional area and a significant decrease in CD. Compared with untreated control dogs, treatment with CAP resulted in a significant reduction of volume fraction of interstitial fibrosis, ODD, and myocyte cross-sectional area and a significant increase in CD. The volume fraction of replacement fibrosis also tended to decrease after treatment with CAP, but the change did not reach statistical significance (Table 3).

**Protein Expression and Other Biochemical Findings**

Changes in protein levels in LV myocardium of NL dogs, untreated HF dogs, and CAP-treated HF dogs for GLUTs, UPCs, CS, mCPT-1, porin, and calsequestrin are shown in Figure 2. There were no changes in expression of calsequestrin and porin among NL dogs, untreated HF dogs, and CAP-treated HF dogs. Compared with NL dogs, dogs in the untreated control group showed a significant reduction in the levels of UCP-2, UCP-3, GLUT-1, GLUT-4, CS, mCPT-1, and SERCA-2a. Compared with untreated controls, treatment with CAP was associated with a significant increase in the expression of all these proteins to near NL levels (Figure 2). Compared with NL dogs, the $V_{max}$ for Ca$^{2+}$-ATPase activity (measured as nmol/mg protein) decreased significantly in the untreated control group and treatment with CAP prevented this decline (Figure 3). These changes were reflected in the affinity (Ka) as well which showed significant increase in the control group that was normalized when treatment with CAP was instituted (Figure 3).

**Discussion**

This is the first study to evaluate the short (1 week)- and long-term (12 weeks) efficacy of a selective partial A1R agonist in HF dogs. The results indicate that 12-week monotherapy with CAP improves LV systolic function and prevents progressive LV enlargement as evidence by a significant improvement in LVEF and significant reduction of LVESV and no significant increase in LVEDV compared with untreated controls. Of interest is the observation of increased LVEF early (1 week) in the course of therapy. This observation suggests that unlike the delayed therapeutic benefits encountered with prototypical drugs used in the treatment of HF, such as β-blockers and angiotensin-converting enzyme inhibitors, the benefits derived from A1R agonism are manifested early in the course of therapy. The improvement of LV systolic function was accompanied by significant increases of SV and CO with a significant decrease of SVR. In the absence of a change in mean aortic pressure and LVESP, the decrease in SVR can be attributed to increased CO. It should be noted, however, that a possible reduction of AoP may have been masked by an increase in CO. Although vasodilation cannot be completely excluded as a contributing factor in improvement of LV systolic function, most, if not all, pharmacological agents that elicit their benefits through vasodilation lower systemic blood pressure, a hemodynamic alteration not seen in the present study after therapy with CAP. Consistent with its partial agonism properties, and unlike side effects seen with full A1R agonist, CAP had no effects on HR and did not at any time during the study trigger atrioventricular block, sedation, or antidiuretic effects. There was no evidence of worsening renal function as evidenced by a lack of an increase of either creatinine or blood urea nitrogen with CAP therapy. The concept that HF patients with renal dysfunction can benefit from selective antagonism of the A1R failed to materialize in large, randomized, placebo-controlled clinical trials.16

The improvement of systolic function seen in the present study cannot be explained on the basis of reduced HR, reduced systemic blood pressure, or an intrinsic improvement in contractility. Therapy with CAP did not induce bradycardia, did not lower systemic pressure, and did not significantly alter the slope of the ESPVR, a load-independent measure of

<table>
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<tr>
<th>Table 2. Treatment Effect, Δ, Between Pretreatment and 12 Weeks in Untreated Control and Capadenoson-Treated Dogs</th>
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<tr>
<td><strong>Parameter</strong></td>
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<tr>
<td>ΔHR, beats per min</td>
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<tr>
<td>ΔmAoP, mm Hg</td>
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<tr>
<td>ΔCO, L/min</td>
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<tr>
<td>ΔLVESV, mm Hg</td>
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<td>Δτ, ms</td>
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<td>ΔLV +dP/dt, mm Hg/s</td>
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<tr>
<td>ΔSVR, dynes/(s·cm$^2$)</td>
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<tr>
<td>ΔEi/Ai</td>
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<td>ΔDT, ms</td>
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<tr>
<td>ΔLV EDWS, g/cm$^2$</td>
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<tr>
<td>ΔSlope of ESPVR, mm Hg/mL</td>
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<tr>
<td>ECG from Holter</td>
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<td>Max HR, beats per min</td>
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<td>Avg HR, beats per min</td>
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<td>Min HR, beats per min</td>
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Data are shown as mean±SEM. Avg indicates average; CO, cardiac output; DT, deceleration time of early mitral inflow velocity; $+dP/dt$, peak rate of change of pressure during isovolumic period; EDWS, LV end-diastolic circumferential wall stress; Ei/Ai, ratio of Ei to Ai; ESPVR, end-systolic pressure-volume relationship; ESP, end-systolic pressure; HR, heart rate; LV, left ventricular; mAoP, mean aortic pressure; Max, maximum; Min, minimum; SVR, systemic vascular resistance; $P$ value, probability control vs capadenoson; and $τ$, time constant of isovolumic relaxation.
contractility or peak LV \(+dP/dt\), a load-dependent measure of isovolumic tension. Because the slope of the ESPVR was based on a small sample size, and because \(+dP/dt\) tended to increase with CAP therapy, one cannot exclude a contractility increase as a contributing factor to the overall benefits on LV function elicited by CAP therapy. Nonetheless, these observations suggest that CAP does not act through negative dromotropic or chronotropic effects.

Although the literature is replete with studies describing the effects of adenosine and its receptors in myocardial ischemia and infarction, to little is known of the effects of adenosine and its receptors in chronic HF. Adenosine and adenosine analogs have been long recognized as in vivo cardioprotective agents and mediators of anti-ischemic preconditioning. These cardioprotective effects are believed to result from activation of downstream effectors, such as protein kinaseC, KATP channel, and some isoforms of mitogen-activated protein kinase and partly by inhibition of adenylate cyclase and reduction of cAMP levels. Given that regional myocardial ischemia and/or hypoxia frequently exist in the failing heart, it is likely that the benefits seen in the present study with CAP reflect reduced cellular injury resulting from the anti-ischemic cardioprotective effects of adenosine A1R activation. Studies have shown that adenosine levels in the setting of HF might act partly through similar signaling cascades, such as adenylylate cyclase inhibition as β-blockers. Long-term (3 months) monotherapy with metoprolol, a selective β1-receptor blocker, in the same animal model of HF used in the present study, was also shown to significantly increase LVEF, although to a lesser extent compared with CAP. The hemodynamic response to β-blockade, however, differs from that seen with the partial A1R agonist. It is well known that in patients with HF, β-blockers reduce HR and induce a negative inotropic effect early in the course of therapy evidenced by reduced LV systolic function before improvement takes place later in the course of therapy. In contrast, results from the present study show that the partial A1R agonist CAP does not lower HR and, contrary to β-blockers, elicits a marked and significant improvement in LVEF as early as 1 week after initiating therapy. These differences argue in favor of additional mechanisms unique to partial A1R agonism that partly drive the observed improvement in LV systolic performance.

Improvement of LV systolic function with CAP may also be the result of improved myocardial energetics elicited through selective activation of the adenosine A1R. In ischemia and infarction, adenosine, acting through its A1R, is known to slow ATP depletion through stimulation of glycolysis, increasing glucose uptake, inhibiting adrenergic stimulation and neutrophil activation, and reducing the generation of free oxygen radicals. The failing myocardium is often described as being energy starved and/or oxygen deprived, suggesting that poor availability of ATP and lack of oxygen may be partly responsible for the characteristic poor LV performance, a signature of the failing heart. The beneficial effects of selective A1R agonism in HF can act to improve energy metabolism of the failing heart via improvement of mitochondrial function and/or energy substrate utilization.

We previously showed that the failing myocardium is characterized by mitochondrial dysfunction evidenced by (1) poor mitochondrial respiration, (2) low mitochondria

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**Table 3. Histomorphometric Findings at the End of 12 Weeks of Follow-up or Therapy in Normal Dogs, in Untreated Control Heart Failure Dogs and in Heart Failure Dogs Treated With Capadenoson**

<table>
<thead>
<tr>
<th></th>
<th>VFRF, %</th>
<th>VFF, %</th>
<th>CD, cap/mm²</th>
<th>ODD, μm</th>
<th>MCSA, μm²</th>
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<tr>
<td>Normal</td>
<td>10.8±2.63</td>
<td>11.3±1.31</td>
<td>2077±46.1 *</td>
<td>11.5±0.51</td>
<td>548±24.4</td>
</tr>
<tr>
<td>HF control</td>
<td>12.3±1.34</td>
<td>15.2±0.63</td>
<td>1775±72.9</td>
<td>14.5±0.44</td>
<td>663±12.3</td>
</tr>
<tr>
<td>HF capadenoson</td>
<td></td>
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Data are shown as mean±SEM. CD indicates capillary density; HF, heart failure; MCSA, myocyte cross-sectional area; ODD, oxygen diffusion distance; VFF, volume fraction of interstitial fibrosis; VFRF, volume fraction of replacement fibrosis.

*P<0.05 vs normal.
†P<0.05 vs HF control.
membrane potential, and (3) abnormal mitochondria membrane permeability transition all of which can lead to poor electron flux through electron transport chain and subsequent reduction of ATP synthesis. Mitochondrial UCPs, in particular, UCP-2 and UCP-3, are transport proteins located in the inner mitochondrial membrane and control the mitochondrial membrane potential and consequently regulate mitochondrial ATP synthesis and the production of reactive oxygen species by the mitochondria. Several studies have shown marked downregulation of UCP-2 and UCP-3 in the failing myocardium. In the present study, we also showed a significant downregulation of UCP-2 and UCP-3 in LV myocardium of untreated HF control dogs. In failing myocardium caused by doxorubicin toxicity, UCP-2 and UCP-3 were significantly decreased and was associated with a significant reduction in mitochondria state-3 and state-4 respiration and ATP synthesis. Several studies have shown that increased expression of UCPs decreases reactive oxygen species production, improves cardiomyocyte survival, and improves contractile function in the setting of ischemia/reperfusion injury. In the present study, long-term therapy with CAP was associated with a near normalization of UCP-2 and UCP-3 protein levels in the LV myocardium.

Selective adenosine A1R agonist can also impact positively on mitochondrial function in HF by modulating the mitochondrial permeability transition pore (mPTP). In addition to providing energy for biological reactions, mitochondria directly regulate cell necrosis and apoptosis through opening of mPTP. We have shown that HF is associated with opening of the mPTP with an attendant increase in the levels of cytochrome c in the cytosol and consequently an increase in cardiomyocyte apoptosis. We also showed that prevention of mPTP opening with cyclosporine A can have marked beneficial effects on mitochondrial respiration and ATP synthesis. Studies by Xiang et al in isolated cardiomyocytes showed that exposure of cardiomyocytes to hypoxia increased both mPTP opening and production of reactive oxygen species while decreasing cell viability and mitochondrial membrane potential. In their study, exposure of the hypoxic

Figure 2. Bar graphs depicting changes in left ventricular myocardium protein levels of various metabolic and sarcoplasmic reticulum proteins in normal (NL) dogs, untreated heart failure control dogs (CON), and dogs with heart failure treated with capadenoson (CAP). Data are shown as mean±SEM. *P<0.05 vs NL; **P<0.05 vs CON. CS indicates citrate synthase; CSQ, calsequestrin; GLUT, glucose transporter; mCPT-1, muscle carnitine palmitoyl transferase-1; SERCA-2a, sarcoplasmic reticulum calcium ATPase-2a; and UCP, uncoupling protein.
Cardiomyocytes to the adenosine A1R agonist 2-chloro-N6-cyclopentyladenosine, blocked the increase in mPTP opening and the production of reactive oxygen species and maintained cell viability and mitochondrial membrane potential under hypoxic conditions.44 These observations of improved mitochondrial function in the form of respiration, membrane potential, and the production of reactive oxygen species and maintained the enzyme responsible for catalyzing the first reaction of the citric acid cycle, is known to be downregulated in HF and is often used as a marker of intact mitochondria.36 Improved ATP synthesis by mitochondria can also account for our observations of normalization of CS levels in LV myocardium of dogs treated with CAP compared with untreated controls. CS, the enzyme responsible for catalyzing the first reaction of the citric acid cycle, is known to be downregulated in HF and is often used as a marker of intact mitochondria.36 Improved ATP synthesis by mitochondria can also account for our observation of increased sarcoplasmic reticulum Ca2+-ATPase activity and affinity after treatment with CAP, a finding consistent with improved sarcoplasmic reticulum calcium cycling and a desired reduction in calcium overload, long recognized as a key maladaptation in HF. The observations made in this study along with observations made by others as outlined above support the concept that in HF, adenosine A1R agonism favorably modulates mitochondrial function and, in doing so, restores availability of ATP to the working myocardium and limit cell injury and loss that may result from excess reactive oxygen species production and programmed cell death. A limitation of the present study is the lack of direct assessment of mitochondrial function in the form of respiration, membrane potential, and opening of permeability transition pores, as well as measurements of ATP synthesis. These measurements must be performed in fresh tissue and are being contemplated for future studies of the effects of A1R agonists in HF.

Consistent with observation by others,36,37 results from the present study point to a significant decrease in key metabolic proteins in untreated HF dogs compared with NL dogs specifically, a reduction in the expression of the glucose transporters, GLUT-1 and GLUT-4, and the regulator of fatty acid oxidation, mCPT-1. These observations are in-line with the concept of reversion of the failing heart to a fetal metabolic phenotype by downregulating adult gene transcripts rather than upregulating fetal gene transcripts.36 In the NL heart, recruitment of the glucose transport proteins, GLUT-1 and GLUT-4, is a cellular mechanism by which the heart increases glucose transport for metabolism in response to increased energy demands. The observed downregulation of GLUT-1 and GLUT-4 is consistent with impaired glucose uptake in HF, a maladaptation that can result in worsening of the HF state.45 In the present study, treatment with a partial adenosine A1R agonist restored protein levels of GLUT-1 and GLUT-4 to near NL levels. Observation in this study also pointed to significant downregulation of mCPT-1 in LV myocardium of untreated HF dogs. Expression of mCPT-1 has been shown to correlate positively with palmitate oxidation suggesting that decreased mCPT-1 protein levels can lead to reduced fatty acid oxidation.46 In the present study, long-term therapy with CAP normalized protein expression of mCPT-1. Restoration of expression of these key metabolic proteins can result in better balance of substrate utilization in the failing heart and, subsequently, improved energy metabolism leading to improved LV pump function.

Global remodeling of the failing LV is invariably accompanied by structural changes at the cellular level characterized by cardiomyocyte hypertrophy, accumulation of collagen in the interstitium termed reactive interstitial fibrosis, reduced CD, and increased ODD. Myocardium of failing heart is associated with structural changes in the form of cardiac hypertrophy and collagen accumulation in the interstitium.14,15,47 These abnormalities favor increased LV stiffness and the development of hypoxia that can lead to progressive worsening of LV function.47 A reduction in interstitial fibrosis and cardiomyocyte hypertrophy, therefore, is likely to reduce LV stiffness and, in doing so, improve passive LV filling. In the present study, CAP treatment prevented the increase in volume fraction of interstitial fibrosis, ODD, and decrease in CD. CAP also decreased myocyte cross-sectional area are markers of cardiac hypertrophy. We have shown that hypoxia of the failing myocardium can be an important mediator of apoptosis through activation of key proapoptotic proteins.48–50 As discussed earlier, hypoxia can also have an adverse impact on mitochondrial function and, in turn, ATP synthesis.44 The long-term use of a partial adenosine A1R agonist in the present study resulted in amelioration of all the cellular markers of structural remodeling suggesting that this approach to therapy for HF can directly or indirectly modulate LV remodeling favorably in addition to improving LV systolic function.

In conclusion, the results of this study support the continued development of selective and partial adenosine A1R agonists for the chronic treatment of HF. Use of CAP in this study was devoid of adverse effects often seen with full A1R agonist. The benefits of this targeted approach to the treatment of HF lies in the ability of partial A1R agonism to afford protection to the failing myocardium by limiting triggers of cell injury and death and by providing the necessary energy to the working myocardium through better energy substrate usage and improved mitochondrial function.

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References
CLINICAL PERSPECTIVE

Adenosine is known to have cardioprotective effects and mediates ischemic preconditioning but its clinical applicability is limited because of significant side effects. Partial adenosine A1-receptor agonists are tailored to deliver beneficial effects of adenosine without producing undesirable side effects because of its high selectivity for A1 receptors. We have used a dog model of microembolization-induced chronic heart failure to study a partial A1-receptor agonist, capadenoson. We found that chronic therapy with this drug resulted in a significant increase in ejection fraction, cardiac output, and stroke volume, and a significant decrease in circulating levels of n-terminal pro–brain natriuretic peptide without affecting heart rate, mean aortic pressure, or renal function. Therapy with capadenoson led to decrease in plasma norepinephrine levels, suggesting its additional antiadrenergic benefit in heart failure. Furthermore, capadenoson treatment resulted in amelioration of all the cellular markers of structural remodeling suggesting that this approach to therapy for heart failure can modulate left ventricular remodeling favorably in addition to improving left ventricular systolic function. These findings suggest that there may be a role of partial A1-receptor agonists in the treatment of patients with heart failure.
Chronic Therapy With a Partial Adenosine A1-Receptor Agonist Improves Left Ventricular Function and Remodeling in Dogs With Advanced Heart Failure
Hani N. Sabbah, Ramesh C. Gupta, Smita Kohli, Mengjun Wang, Sharad Rastogi, Kefei Zhang, Katja Zimmermann, Nicole Diedrichs and Barbara E. Albrecht-Küpper

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