Neurohormonal antagonism in heart failure; beneficial effects of vasopressin V$_{1a}$ and V$_2$ receptor blockade and ACE inhibition

Mareo Naitoh, John Risvanis, Leanne C. Balding, Colin I. Johnston, Louise M. Burrell*

Department of Medicine, University of Melbourne, Austin and Repatriation Medical Centre, Heidelberg, Victoria 3084, Australia

Received 24 September 2001; accepted 27 December 2001

Abstract

Objective: To assess the long-term efficacy of vasopressin (AVP) V$_{1a}$ and V$_2$ receptor blockade with conivaptan, alone and in combination with angiotensin converting enzyme (ACE) inhibition on blood pressure, metabolic and neurohormonal parameters, and cardiovascular structure in a rat model of congestive heart failure (CHF).

Methods: CHF was induced by left coronary artery ligation. CHF rats received conivaptan (1 mg/kg/day), ACE inhibition (captopril, 50 mg/kg/day), conivaptan and captopril (Combination) or vehicle for 4 weeks. Blood pressure was measured weekly, metabolic caging studies performed at 25 days, and rats killed and blood and tissue collected after 4 weeks treatment.

Results: Combination treatment lowered blood pressure ($P < 0.01$), and conivaptan and Combination caused an aquaresis ($P < 0.01$). Combination decreased plasma natriuretic peptide ($P < 0.05$), reduced left and right ventricular mass ($P < 0.01$) and lung mass ($P < 0.05$).

Conclusions: In CHF, blockade of vasopressin V$_{1a}$ and V$_2$ receptors was associated with increased water excretion, and the combination of conivaptan with ACE inhibition was the only treatment to reduce blood pressure, natriuretic peptide and pulmonary congestion. These results suggest conivaptan may be a useful addition to ACE inhibitors in the management of vasoconstriction and fluid retention that characterizes CHF. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: ACE inhibitors; Blood pressure; Heart failure; Natriuretic peptide

This article is referred to in the editorial by S.R. Goldsmith (pages 13–15) in this issue.

1. Introduction

In congestive heart failure (CHF) short term activation of neurohormonal systems preserves circulatory homeostasis and maintains arterial pressure but when present in chronic excess, these ‘compensatory’ systems play a role in the development and progression of CHF [1]. Early compensatory mechanisms include activation of the sympathetic nervous system, natriuretic peptides, the renin–angiotensin system and levels of arginine vasopressin (AVP) also rise [2]. Vasopressin has adverse effects in heart failure to increase peripheral resistance via constrictor actions at the V$_{1a}$ receptor [3,4] and contribute to water retention through effects at the renal V$_2$ receptor [5]. Thus, blockade of the AVP system may prove a useful adjunct to standard therapy in CHF, as V$_{1a}$ receptor blockade offers an additional method of vasodilation whilst V$_2$ receptor blockade has aquaretic effects which are beneficial in symptomatic CHF [6,7].

In heart failure in rat, rabbit, pig and dog, acute studies indicate that selective V$_{1a}$ or V$_2$ receptor blockade has short-term metabolic, hormonal responses or hemodynamic benefits [3,8,9]. Relatively few studies have assessed the combination of V$_{1a}$ and V$_2$ receptor blockade in CHF, and all have been acute in nature [9–11]. Two studies have assessed the combination of V$_{1a}$ receptor blockade and angiotensin converting enzyme (ACE) inhibition [3,4], but to date it is not known whether V$_2$ receptor blockade, or V$_{1a}$ and V$_2$ receptor blockade plus ACE inhibition is of additional benefit in CHF. Such ‘combination’ studies are of importance, as ACE inhibitors are standard therapy in heart failure given their benefits to slow progression of...
myocardial dysfunction, alleviate symptoms and reduce morbidity and mortality [12].

Conivaptan (YM087) is the first orally effective nonpeptide antagonist of both V$_{1a}$ and V$_2$ receptors [13,14], and in vitro antagonizes the action of AVP in vascular smooth muscle cells indicating V$_{1a}$ receptor antagonism, and blocks AVP induced cAMP production by renal epithelial cells indicating V$_2$ receptor antagonism [15]. In vivo, conivaptan blocks the pressor response to AVP in dogs [13] indicating V$_{1a}$ receptor antagonistic actions whilst its aquaretic actions in the dog and rat indicate significant inhibition of V$_2$ receptor function [13,14].

As it is likely that vasopressin receptor blockade would be used as an adjunct to blockade of the renin–angiotensin system rather than as monotherapy in the management of heart failure, this study was designed to assess the long-term efficacy of V$_{1a}$ and V$_2$ receptor blockade with conivaptan, alone and in combination with ACE inhibition. In particular the effect of treatment on blood pressure, metabolic and neurohormonal parameters, and cardiovascular structure in the coronary artery ligation model of heart failure were assessed [5,16].

2. Methods

Experimental procedures were approved by the Austin Hospital Animal Research Ethics Committee and performed according to the National Health and Medical Research Council of Australia Guidelines for animal experimentation.

Conivaptan [YM087, 4’-(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-d](1)benzoazepine-6-carbonyl)-2-phenylbenzamidile] monohydrochloride) was a gift from Yamanouchi (Tokyo, Japan). Captopril was purchased from Sigma (MO, USA). Female Sprague–Dawley rats (150–200 g) from the Austin Biological Research Labs., (Austin, USA) and Repatriation Medical Centre were housed at 23–25 °C in a 12 h light–dark cycle with access to a standard rat chow (0.4–0.6% NaCl) and normal water ad libitum.

2.1. Experimental design

The aim of the present study was to compare in experimental CHF, the effect of 4 weeks treatment with V$_{1a}$ and V$_2$ receptor blockade, and combined AVP receptor blockade and ACE inhibition. The dose selected for conivaptan was based our previous work [14] in which oral conivaptan (0.1–3 mg/kg) dose dependently inhibited vasopressin binding to liver V$_{1a}$ and kidney V$_{1a}$ and V$_2$ receptors over 24 h. The 1 mg/kg dose of conivaptan was chosen as this dose caused blockade of both V$_{1a}$ and V$_2$ receptors measured using autoradiography and binding techniques [14]. The dose of captopril was chosen as this dose attenuates cardiac remodeling and improves survival in this model of CHF [16–18].

Left ventricular free-wall myocardial infarction was induced in rats by ligation of the proximal left anterior descending artery [5,6,16,19]. Sham operated (Control) rats underwent an identical operation but the suture was not tied. At 24 h postsurgery, surviving rats were randomized to one of four treatment groups; vehicle (water), conivaptan (1 mg/kg/day), captopril (50 mg/kg/day in two divided doses), or conivaptan plus captopril (Combination). Drugs were given by oral gavage for 4 weeks. Control rats received vehicle.

Body weight and systolic blood pressure (SBP) were measured weekly between 90 and 120 min after dosing by one observer blinded to the treatment of each rat. SBP was measured by the indirect tail-cuff technique (38L flatbed recorder, model 229 Ampli®er, IITC Life Science, Woodland Hills, CA, USA) in conscious, lightly restrained rats. During the fourth week of treatment, a randomly selected subset of rats (n=6–9 per group) were placed in metabolic cages and food and fluid intake, as well urine volume, sodium and osmolality were assessed. After 4 weeks treatment, rats were killed by decapitation and trunk blood collected into prechilled lithium heparin tubes for measurement of plasma osmolality, sodium and plasma AVP, and tubes containing EDTA/aprotinin (kallikrein inhibitor 500 U/ml) for the measurement of atrial natriuretic peptide (ANP) and plasma renin activity (PRA). The left ventricle and interventricular septum (LV) were dissected from the heart; weighed and fixed in 10% buffered formalin. The right ventricle (RV) and lungs were weighed. To determine infarct size the LV was sectioned at four levels from the base to the apex, paraffin fixed and sections cut and stained with Masson’s trichrome. The mean epicardial and endocardial scar circumference was compared to total left ventricular circumference to calculate total infarct size [16]. Rats with a subendocardial infarct or infarct size of less than 20% were excluded from analysis.

2.2. Analytical methods

Plasma AVP, ANP and PRA were measured by radioimmunoassay as previously described [20–22]. Urine and plasma osmolality were measured using a Wescor vapor pressure osmometer 5100C (Logan, UT, USA). Urine sodium was measured using an ion-selective electrode (ILyte, Instrumentation Lab., Italy).

2.3. Statistics

The results are presented as means±S.E.M. Longitudinal SBP and body weight were compared between treatment groups by ANOVA for repeated measures followed by posthoc analysis using ANOVA and Fisher’s PLSD test when appropriate. The metabolic, plasma, hormonal and tissue weight data were analyzed by ANOVA and Fisher’s
PLSD test. Significant differences were obtained when $P<0.05$.

### 3. Results

Cages were examined twice daily to assess the health of the animals. Of 160 rats operated on to tie off the coronary artery, 72% ($n=115$) were alive at 24 h and were randomized to vehicle ($n=29$), conivaptan ($n=29$), captopril ($n=31$), or conivaptan plus captopril (Combination, $n=26$). All sham operated rats survived ($n=14$).

In the CHF group, rats with a subendocardial infarct or infarct size of less than 20% were excluded from analysis ($n=53$). Six CHF rats (vehicle, $n=3$; conivaptan, $n=1$; captopril, $n=1$; Combination, $n=1$) died during the course of the study and were also excluded from the analysis. Results are thus reported on 56 infarct rats (vehicle, $n=11$; conivaptan, $n=17$; captopril, $n=16$; Combination, $n=12$) and 14 vehicle treated, non-infarcted rats (Control). The average infarct size was 39–43% with no significant difference among treatment groups (Table 1).

#### 3.1. Weight and systolic blood pressure

Baseline body weight was similar in all groups (182±2 g), and all rats gained weight during the study period ($P<0.01$). At week 4, rats treated with captopril and Combination therapy had reduced body weight compared to vehicle (Captopril, $P<0.05$, Combination, $P<0.01$, Table 1), with a greater reduction with Combination treatment compared to captopril ($P<0.05$). There was no significant difference in food intake (data not shown) between the groups.

Baseline systolic blood pressure was similar in all groups, but 4 weeks after infarction blood pressure was lower in vehicle treated CHF compared to Control rats ($P<0.01$). At week 4, blood pressure was even lower on captopril ($P=0.055$) and significantly lowered by Combination treatment ($P<0.01$) compared to vehicle (Fig. 1).

#### 3.2. Metabolic parameters

There were no differences in urine osmolality, output or sodium excretion between Control and CHF rats (Table 1, Fig. 2). In CHF, after 4 weeks treatment, urine osmolality was reduced by conivaptan and Combination treatment ($P<0.01$) (Table 1). Conivaptan alone or in combination with captopril significantly increased urine volume. There was a nonsignificant trend to increased urinary sodium excretion with conivaptan ($P=0.054$) and Combination ($P=0.066$) (Fig. 2). Captopril had no significant effect on any renal parameter. In CHF, plasma osmolality, AVP and PRA were similar to values in Control rats (Table 1). Conivaptan had no effect on plasma osmolality but increased plasma AVP ($P<0.05$), whilst PRA was increased by captopril and Combination treatment ($P<0.01$). Plasma ANP was significantly increased in CHF compared to Control rats ($P<0.05$), and was reduced by Combination treatment alone ($P<0.05$).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Parameters in Control and CHF rats after 4 weeks treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>CHF</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>0</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>230±2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>120±2</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td>1536±91</td>
</tr>
<tr>
<td>Urine sodium (μmol/min/100 g)</td>
<td>0.47±0.10</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/kg)</td>
<td>284±2</td>
</tr>
<tr>
<td>Plasma AVP (pmol/l)</td>
<td>5.8±0.5</td>
</tr>
<tr>
<td>PRA (nmol AI/l/h)</td>
<td>5.2±1.0</td>
</tr>
<tr>
<td>Plasma ANP (pmol/l)</td>
<td>25±4</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.; $n=14–18$ for infarct size; $n=14–17$ for body weight and blood pressure; $n=6–9$ for urine data; $n=6–17$ for plasma osmolality and AVP, $n=10–17$ for plasma PRA and ANP.

$^*$, $P<0.05$; $^\dagger$, $P<0.01$ Control vs. CHF vehicle; $^*$, $P<0.05$, $^{**}$, $P<0.01$ CHF vehicle vs. CHF treatment; $^*$, $P<0.05$ Combination vs. Captopril.
Fig. 2. Urine volume and sodium excretion in Control (n=6) and CHF rats (n=6–9 per group) measured on Day 25 of treatment. *, P<0.05; **, P<0.01 CHF vehicle vs. CHF treatment; †, P<0.05, CHF captopril vs. Combination.

Table 2
Organ mass in Control and CHF rats after 4 weeks of V₁a and V₂ receptor blockade using conivaptan and/or ACE inhibition

<table>
<thead>
<tr>
<th></th>
<th>Control Vehicle</th>
<th>CHF Vehicle</th>
<th>Conivaptan</th>
<th>Captopril</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle (g/kg)</td>
<td>2.28±0.03</td>
<td>2.78±0.09</td>
<td>2.71±0.08</td>
<td>2.51±0.08</td>
<td>2.46±0.06</td>
</tr>
<tr>
<td>Right ventricle (g/kg)</td>
<td>0.80±0.01</td>
<td>1.2±1.1</td>
<td>0.84±0.08</td>
<td>0.97±0.09</td>
<td>0.72±0.05</td>
</tr>
<tr>
<td>Atria (g/kg)</td>
<td>0.39±0.01</td>
<td>0.80±0.01</td>
<td>0.80±0.01</td>
<td>0.67±0.01</td>
<td>0.69±0.01</td>
</tr>
<tr>
<td>Lung (g/kg)</td>
<td>5.9±0.4</td>
<td>8.6±1.0</td>
<td>7.8±0.7</td>
<td>6.9±0.5</td>
<td>6.2±0.3</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.; n=13–18.
††, P<0.01 Control vs. CHF vehicle; *, P<0.05; **, P<0.01 CHF vehicle vs. CHF treatment; †, P<0.05 Combination vs. Captopril.

3.3. Cardiovascular structure

CHF rats were characterized by increased relative LV, RV, atria and lung mass compared to Control rats (all P<0.01) (Table 2). As expected in CHF, captopril reduced LV (P<0.01) and RV mass (P<0.05) compared to vehicle (Fig. 3). Conivaptan did not influence LV mass but significantly reduced RV mass (P<0.01), and in combination with captopril reduced both LV and RV mass (P<0.01), as well as reducing lung mass (P<0.05) (Table 2).

Relative atria mass was not significantly changed by any treatment, but was lower in the captopril and Combination groups.

4. Discussion

The long term response to vasopressin V₁a and V₂ receptor blockade in experimental CHF has not previously been investigated, and this is also the first study to
compare and combine $V_{1a}$ and $V_2$ receptor blockade and ACE inhibition in experimental heart failure. The major findings of this study in heart failure were that conivaptan, a $V_{1a}$ and $V_2$ receptor antagonist increases water excretion, and in combination with an ACE inhibitor, lowers blood pressure, body weight, pulmonary congestion and ANP secretion.

4.1. Postinfarction heart failure model

The model of myocardial infarction induced heart failure used in this study results in hemodynamic alterations and neurohormonal changes [5,17] similar to those seen in patients with anterior myocardial infarction and results using this model have clinical implications [12]. All infarcted rats in this study had histological verification of infarct sizes, and all showed signs of pulmonary congestion. We have previously shown that heart failure in this model is characterized by cardiac remodeling with LV cavity dilation, hypertrophy of surviving myocardium and impaired systolic function [23]. With regard to neurohormonal activation, this is a compensated model of heart failure with normal renin activity and plasma vasopressin levels, but elevated natriuretic peptide levels [5,19].

4.2. Metabolic and neurohormonal effects of conivaptan

After 1 month of treatment, the renal response to conivaptan was characterized by increased urine volume and reduced urine osmolality, indicating significant inhibition of renal $V_2$ receptor function. There was trend to increased urinary sodium excretion by conivaptan but this did not reach significance. In long-term (6 month) CHF studies, the effect of conivaptan on sodium excretion was equivalent to vehicle (Vehicle, 0.58 ± 0.03 μmol/min/100 g, Conivaptan, 0.47 ± 0.05 μmol/min/100 g, n = 10/group, unpublished data). These results confirm acute studies in dogs [10,13] and rats [14] using conivaptan, and extend them to demonstrate the aquaretic effects of conivaptan are maintained long term in heart failure. The renal effects of conivaptan were not accompanied by an increase in plasma osmolality, which may reflect the fact that rats had free access to water.

Treatment with nonpeptide $V_2$ receptor blockers such as OPC-31260 [5,9] causes an aquareasis and increases plasma vasopressin [5], and this was also seen with conivaptan. As urine volume was similar with conivaptan and combination therapy, the increase in plasma AVP was not able to overcome $V_2$ receptor blockade. A concern with selective $V_2$ receptor blockers is that their clinical efficacy to reduce preload is offset by unopposed activation of the $V_{1a}$ receptor. However, conivaptan has the advantage that any contribution of elevated vasopressin to systemic vasoconstriction would be prevented by its $V_{1a}$ receptor blocking properties [6]. Interestingly, plasma vasopressin was not significantly elevated with combined treatment with conivaptan and captopril suggesting improved vasopressin metabolism [24] as a consequence of improved renal function.

Vasopressin suppresses renin release [25], and improved renal flow may also explain the lack of rise in PRA with vasopressin receptor antagonism. Atrial natriuretic peptide which is elevated in proportion to the degree of left ventricular dysfunction [26] and correlates with prognosis, was reduced only by combination treatment with conivaptan and captopril, which suggests cardiac function may have improved more with the combination than with ACE inhibition alone. Although cardiac function was not specifically assessed in this study, the cardiac benefits of conivaptan in the dog pacing model of heart failure to increase cardiac output and decrease left ventricular end-diastolic pressure would also be expected to reduce ANP secretion [10].

4.3. Blood pressure and cardiovascular structural effects of conivaptan

Combination treatment with conivaptan and captopril led to a small but significant fall in blood pressure in CHF, and as the fall with captopril alone did not reach significance, this additional effect may represent $V_{1a}$ receptor blockade. Vasopressin receptor blockade was not directly assessed in this study but our previous binding and in vitro autoradiography studies show that conivaptan (1 mg/kg) inhibits AVP binding at both $V_{1a}$ receptors in liver and kidney [14], and renal $V_2$ receptors. In addition, autoradiographic data from ongoing studies in rat heart failure indicate that both $V_{1a}$ and $V_2$ receptors are blocked following long-term (6 month) treatment with conivaptan (unpublished data). Further evidence of the $V_{1a}$ receptor blocking efficacy of conivaptan is shown by its ability to inhibit the pressor response to exogenous vasopressin [13], and to decrease mean arterial pressure total and peripheral resistance in heart failure [10].

Few studies have investigated combined $V_{1a}/V_2$ receptor and renin–angiotensin system blockade in heart failure. Experimental and clinical trials show ACE inhibitors slow the deterioration of the failing heart and improve long term survival partly through reversal of the neurohormonal activation, and also through attenuation of remodeling [18]. In real terms, the modest effect of ACE inhibitors on mortality and the continued remodeling even in the face of ACE inhibition [1] highlights the need for additional therapy. Short-term studies in rabbits with acute left ventricular failure [3] reported the combination of a peptide $V_{1a}$ receptor antagonist and an ACE inhibitor had additive effects to increase cardiac output and decrease both blood pressure and peripheral resistance [3], but it is not clear whether these short-term improvements persist or are associated with attenuation of ventricular remodeling.

In this study, conivaptan had beneficial effects to regress right ventricular hypertrophy, a marker of overload hy-
pertrophy which may be due to reduced preload due to increased urine output, and/or direct cardiac effects. The heart is known to contain \( V_{1a} \) receptors [27], and AVP is now recognized to be synthesized in the heart and vasculature where it acts as a paracrine hormone [28,29]. Thus vasopressin may serve as both a circulating and as a local tissue hormone system in an analogous manner to the renin–angiotensin system and may have deleterious effects on the heart when present in chronic excess. Vasopressin causes hypertrophy in both isolated perfused hearts and in cardiomyocytes via the \( V_{1a} \) receptor, and this effect can be blocked by selective \( V_{1a} \) receptor antagonism using OPC-21268 [30] or combined \( V_{1a}/V_{2} \) receptor antagonism using conivaptan [31]. In addition, de novo synthesis of AVP (message and protein) in pressure-overloaded rat hearts causes coronary vasoconstriction and impaired relaxation that can be prevented by \( V_{1a} \) receptor blockade [28].

Most recently, the effect of a single intravenous dose of conivaptan in patients with symptomatic heart failure taking ACE inhibitors was studied [32]. Compared to placebo, conivaptan increased urine output, and reduced pulmonary capillary wedge pressure and right atrial pressure. Although it is not clear whether the favorable cardiac effects of conivaptan are simply due to increased urine output, the lack of effect of conivaptan on vascular resistance, and the lack of similarity between pulmonary capillary wedge pressure changes and urine output does suggest that direct cardiac \( V_{1a} \) receptor antagonism may play a role in the benefits observed.

The design of this study in rats did not allow the precise role of \( V_{1a} \) versus \( V_{2} \) receptor blockade on the cardiac changes to be distinguished. However, we have previously examined the effect of the selective \( V_{2} \) receptor blocker, OPC-31260 in the same model of heart failure and found no effect on cardiac mass despite significant aquaresis [5]. Taken together these data suggest that direct cardiac \( V_{1a} \) receptor blocking properties of conivaptan may be responsible for the benefits observed, but further investigations comparing with selective \( V_{1a} \) receptor blockade will be needed to clarify this point.

We also found that in combination with captopril, conivaptan had the additional benefit over selective ACE inhibition of reducing fluid overload as suggested by the reduction in body weight, lung mass and plasma ANP. In practice, ACE inhibitors are used with diuretics to relieve congestive symptoms, although this causes further stimulation of the renin–angiotensin system and sympathetic nervous system which may limit long-term efficacy [33] and contribute to increased risk of arrhythmic death [34]. Studies in CHF have shown that \( V_{2} \) receptor antagonism has no adverse effects on survival [5], and future studies are needed to assess whether neurohormonal antagonism combining blockade of the vasopressin and the renin–angiotensin system will result in improved survival in heart failure.

To summarize, in experimental CHF, blockade of vasopressin \( V_{1a} \) and \( V_{2} \) receptors with conivaptan was associated with increased water excretion. In combination with an ACE inhibitor, conivaptan reduced blood pressure, pulmonary congestion and plasma natriuretic peptide levels. These results suggest that vasopressin receptor blockade may be useful in the treatment of heart failure as an adjunct to ACE inhibition in the management of vasoconstriction and fluid retention that characterizes heart failure.

Acknowledgements

This work was supported by the National Health and Medical Research Council of Australia. Dr. Leanne Balding was supported by a CardioVascular Lipid Research Grant (Pfizer).

References

[13] Yatsus T, Tomura Y, Tahara A et al. Pharmacological profile of...


