Define in Humans With Compensated CHF and Renal Dysfunction, the Modulating Action of Chronic AT1 Receptor Blockade in Addition to ACE Inhibition on Cardiorenal and Humoral Function

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1R01 HL084155-01A2 Specific Aims 2: Define in humans with compensated CHF and renal dysfunction, the modulating action of chronic AT1 receptor blockade in addition to ACE inhibition on cardiorenal and humoral function

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ABSTRACT

The broad objective of this protocol is to advance our understanding of the pathophysiological mechanisms of **human Cardiorenal Syndrome (CRS)** with a specific emphasis upon **the biological interaction between diuretic therapy, the renin-angiotensin-aldosterone-system (RAAS) and cyclic 3′-5′-guanosine monophosphate (cGMP) pathway.**

Chronic heart failure (CHF) as a result of left ventricular systolic dysfunction is a clinical syndrome with high mortality and morbidity. Renal dysfunction is a common and progressive complication of CHF and despite growing recognition of the frequent presentation of combined cardiac and renal dysfunction, or "**Cardiorenal Syndrome (CRS)**", its underlying pathophysiology is not well understood, with a lack of consensus as to its appropriate management. The CRS can be classified into 2 broad categories: 1) patients with compensated CHF and impaired renal function; and 2) patients with decompensated CHF and worsening renal function. Studies have established that renal dysfunction is one of the most important prognostic indicators in patients with CHF, suggesting that it is a manifestation of and/or exacerbating factor for left ventricular dysfunction. In hospitalized patients with decompensated CHF, worsening renal function is even more important than baseline renal function for predicting adverse outcomes.

Diuretics are effective and necessary to relieve congestion in CHF, and some studies have reported that in CHF patients with congestion, diuretic therapy may have favorable neurohumoral and hemodynamic effects. In contrast, recent reports have suggested potential deleterious consequence of chronic diuretic therapy on the progression of CHF, specifically furosemide that is the most widely used diuretic in CHF. Others and we have reported that in compensated CHF patients, furosemide therapy has deleterious neurohumoral and renal hemodynamic effects such as renal vasoconstriction and activation of the RAAS. More importantly we demonstrated that angiotensin II receptor (AT1) blockade prevented the detrimental renal effects of diuretics in these compensated CHF patients. In decompensated CHF patients who present with the combination of worsening renal function, volume overload, and diuretic refractoriness, the management of cardiorenal dysfunction is extremely challenging, and effective therapies are lacking.

The main objective of this study is to translate the findings of the applicant's study in experimental CHF to human CHF and determine the modulating actions of chronic AT1 receptor blockade in addition to ACE inhibitor, beta-blocker and furosemide on cardiorenal and humoral function in humans with compensated CHF and renal dysfunction. Preliminary studies completed by the applicant in experimental CHF have shown improvement in left ventricular function and enhanced renal function with AT1 receptor blockade. Furthermore, the applicant also completed a study in human compensated CHF demonstrating that acute AT1 blockade prevented the detrimental renal effects of furosemide therapy.

Hypothesis: Chronic AT1 receptor blockade in subjects with compensated CHF and renal dysfunction will improve renal function with increased sodium excretion, glomerular filtration rate and effective renal plasma flow and renal function reserve as compared to the response of placebo-treated subjects.

This will be a **double-blinded placebo controlled study design**. Subjects will be randomized to the Chronic AT1 receptor blockade or to the placebo group. We will compare the effect of 12 weeks of Chronic AT1 receptor blockade with Candesartan (n=38) or placebo (n=38) on cardiorenal and humoral in subjects with compensated CHF and renal dysfunction

BACKGROUND AND SIGNIFICANCE

Chronic heart failure (CHF) secondary to left ventricular systolic dysfunction is a clinical syndrome with significant mortality and morbidity despite recent advances in the understanding of the pathophysiology and treatment. The latest figures from the American Heart Association reports a prevalence of 5 million with incidence of 550,000 cases, annual mortality of 52,000 and a total cost of 28 billion dollars in healthcare expense per year in the US.

Cardiorenal Syndrome

Renal dysfunction is a common and progressive complication of CHF and despite growing recognition of the frequent presentation of combined cardiac and renal dysfunction, or "**Cardiorenal Syndrome (CRS)**", its underlying pathophysiology is not well understood, with a lack of consensus as to its appropriate management. The CRS can be classified into 2 broad categories: 1) patients with compensated CHF and impaired renal function; and 2) patients with decompensated CHF and worsening renal function.

Recent studies have established the prognostic importance of renal function in both patients with compensated and decompensated CHF. A multivariate analysis of the patients in the second prospective randomized study of Ibopamine on mortality and efficacy (PRIME) by Hillege et al[1] demonstrated that estimated glomerular filtration rate (GFR) is the most powerful predictor of mortality, exceeding functional status and ejection fraction (EF). Furthermore, a retrospective analysis of the studies in left ventricular dysfunction (SOLVD) treatment trial and SOLVD prevention trial by Dries et al[2] confirmed that estimated GFR as an important determinant of survival. Importantly, Dries et al demonstrated that a mild reduction of estimated GFR had an impact on survival even in patients who were asymptomatic. In patients who are hospitalized with decompensated CHF, worsening renal function is also associated with worse outcome as reported by Gottlieb et al[3] and Smith et al[4] in 2 separate studies. In both studies, an increase in plasma creatinine of 0.2-0.3 mg/dL predicated a worse outcome. Various studies have estimated that 25-30% of patients hospitalized for decompensated CHF has worsening of renal function leading to prolonged hospitalization increased morbidity and mortality.[5-7] The acute decompensated heart failure national registry (ADHERE) database enrolled 160,000 non-selected patients admitted to the 281 participating hospitals for acute decompensated heart failure. In this database, more than 70% of the patients have moderate renal dysfunction as defined by estimated GFR of less than 50 ml/min m^2. Using the classification and regression tree analysis on the ADHERE database, inpatient mortality risk can be estimated by measuring serum creatinine, blood urea nitrogen (BUN) and systolic blood pressure. It has been determined that patients with BUN $\geq$ 43 mg/dL, systolic blood pressure of < 115 mmHg and serum creatinine of $\geq$ 2.75 mg/dL had a 10 fold increase in inpatient mortality.[8]

Pathophysiology of Cardiorenal Syndrome

The underlying pathophysiology of cardiorenal syndrome is not well understood; it is most likely multifactorial and complex. Decreased renal perfusion can lead to renal insufficiency; this may be caused by decreased cardiac output and neurohumoral mediated vasoconstriction by the renin-angiotensin-aldosterone system (RAAS).[9] Recent evidence suggests that both the natriuretic peptide (NP) and nitric oxide (NO) cyclic GMP pathways are impaired in CRS and may contribute to the renal dysfunction.[10,11] Renal cGMP generation, which is calculated as follows: [urine cGMP X urine flow] – [plasma cGMP X GFR}, is a marker of renal responsiveness to the NP and NO.[12] Furthermore, studies have proposed the assessment of renal functional reserve (RFR), that is, the glomerular vasodilatory response to amino acid infusion (AA), as an index of the intrarenal balance between vasocontricting hormones such as angiotensin II and vasodilators such as the NPs and NO.[13,14] Under normal conditions, AA induces a decrease of renal vascular resistances and a consequent increase of GFR. On the other hand, attenuated RFR has been observed in experimental models of renal damage and humans with hypertension, diabetes, and glomerulonephritis.[15] Volpe et al demonstrated elegantly that in human asymptomatic left ventricular dysfunction, RFR is attenuated despite preserved baseline GFR, suggesting that RFR may be an early indicator of renal hemodynamic disruption.[16] RFR has also been described as an indicator of the workload per nephron and may be useful to assess the progression renal dysfunction. To date, no studies have assessed the RFR in patients with compensated CHF and renal dysfunction.

Another cause of renal dysfunction in CHF may be due to elevated venous pressure. This was elegantly demonstrated in a study by Firth et.al[17] who showed that as central venous pressure increased, GFR decreased despite the fact that cardiac output and mean arterial blood pressure was maintained. GFR improved when venous pressure returned to normal. We have recently completed a prospective study to assess the neurohumoral and renal hemodynamic profile in hospitalized patients with decompensated CHF associated with the development of CRS. (see Preliminary studies). Our findings suggest that indeed the combination of pronounced activation of RAAS with decreased renal perfusion pressure (estimated by mean arterial pressure minus central venous pressure) and a relative deficiency of the NPs predisposes to the development of CRS.

Among heart failure patients, several clinical features are more common in those who develop worsening renal function: On average, they are older and have a greater prevalence of prior heart failure, renal dysfunction, diabetes, and hypertension. Furthermore, a study by Butler et al [5] demonstrated that the use of calcium channel blockers and higher loop diuretics doses was associated with higher risk of developing worsening renal failure in patients hospitalized with acute decompensated CHF.

Treatment of Patients with Cardiorenal Syndrome

The role of diuretics, especially furosemide in the management of patients with decompensated CHF and cardiorenal syndrome remains controversial. Studies have suggested that aggressive diuresis with high diuretic doses have been associated with worsening renal function and increased mortality. However it is unclear if diuretics are causally related to increased renal dysfunction and mortality risk.[19] It is likely that diuretic resistance and concomitant worsening renal dysfunction necessitate high doses of diuretics, which are a marker rather than a mechanism for poor outcomes.

Gaps in our Knowledge

The cardiorenal syndrome represents an ominous and frequent development in the natural history of chronic heart failure, yet our understanding of the underlying mechanisms remains rudimentary, and we lack effective therapies. A working group on cardio-renal connections in heart failure convened by the National Heart, Lung and Blood Institute in August 2004 stated in the executive summary that ***"It is clear that our current understanding of cardio-renal connections is inadequate to explain many of the clinical observations in heart failure or to direct its therapy. Further investigation is required to elucidate the pathways by which integration of the cardiovascular and renal systems effectively maintains volume regulation in order to develop effective therapies." [20]***

Diuretics Therapy and the RAAS in CHF

Diuretics are effective in relieving symptoms and edema in CHF. The 4 main classes of diuretics are loop, thiazide, potassium sparing and carbonic anhydrase.[21] Loop diuretics used for CHF include furosemide and torasemide.[22] Recent studies have suggested that torasemide has anti-aldosterone actions and may be safer than furosemide[23-25]. However furosemide is used most widely in both the outpatient therapy of CHF patients and in patients hospitalized for acute decompensated CHF.[22] As such, we will focus on furosemide for this application. While thiazide diuretics have been shown in randomized controlled studies to reduce morbidity and mortality in patients with hypertension[26], furosemide has not been shown to improve outcome in CHF. Recent preclinical studies and retrospective clinical studies have questioned the safety of furosemide use in CHF. McCurley et al demonstrated in a porcine model of CHF that furosemide use resulted in acceleration of both contractile and metabolic features of CHF, including left ventricular systolic dysfunction, elevated aldosterone and altered calcium handling[27]. A retrospective analysis of the studies of left ventricular dysfunction (SOLVD) database reported that the use of potassium sparing diuretic was associated with improved outcome as compared to non-potassium sparing diuretics.[28] We have also demonstrated in a canine model of mild/compensated CHF that chronic furosemide therapy resulted in activation of RAAS and the development of renal resistance to exogenous NPs. (see Preliminary studies)

In the congested patient with LV systolic dysfunction, furosemide is extremely effective in relieving symptoms, reducing intracardiac pressures, and improving cardiac performance. In non-congested patients, administration of furosemide may result in disparate vascular and hemodynamic effects. In an elegant study by Ikram et al[29], they assess the effects of acute furosemide in congested patients with LV systolic dysfunction and subsequently assess the effects of chronic furosemide in the same patients when they are non-congested.

They reported that in congested patients, administration of furosemide resulted in diuresis and improved hemodynamics in the absence of major changes in the RAAS. However, in non-congested patients, the diuretic and hemodynamic responses to furosemide declined whereas the RAAS was activated. Francis et al [30] observed that the administration of intravenous furosemide to patients with CHF on chronic diuretic therapy resulted in an increase in left ventricular filling pressures, a fall in the stroke volume index, and deterioration in pump function. We have also reported recently that the administration of oral furosemide in compensated CHF patients have detrimental renal vascular and neurohumoral effects.[31] (See Preliminary studies) More importantly, we demonstrated that acute AT1 receptor blockade prevented the detrimental renal hemodynamic effects with suppression of aldosterone. Previous studies have also established beneficial renal hemodynamic and excretory function of AT1 receptor blockade in experimental models of CHF.[32] Vople et al demonstrated that 6 weeks of AT1 blockade improve renal functional reserve in patients with mild CHF.[16] It is now known that inhibition of ACE by ACE-inhibitors is neither uniform nor sustained, angiotensin II and aldosterone levels may rise again despite chronic ACE-inhibitor therapy.[33] We have preliminary result suggesting that in patients with compensated CHF on ACE inhibitor therapy and diuretics, plasma angiotensin II levels are still elevated.[34] (See Preliminary studies) Thus, it is possible that noxious effects of diuretics on the RAAS may hence persist despite ACE-inhibitor therapy and may affect prognosis adversely. To date, the renal effects of AT1 blockade in patient with compensated CHF in addition to ACE inhibition has not been assessed.

Gaps in our knowledge

The renal effect of AT1 blockade in compensated patient who is already on ACE-inhibitors and beta-blockers remains undefined.

Significance

Our proposed studies will advance our knowledge of the integrated cardiorenal and humoral physiology in patients with cardiorenal syndrome. Strategies that improve renal function in human cardiorenal syndrome may favorably affect clinical outcomes in this group of CHF patients, who have high morbidity and mortality and no proven effective therapy.

PRELIMINARY STUDIES

To understand the biological interaction between furosemide therapy, RAAS and cGMP pathway, the applicant has completed both laboratory-based studies in canine models of experimental compensated CHF and Overt CHF with cardiorenal syndrome and more importantly Clinical Research Unit (CRU) based patient orientated research studies and case control human studies, which are the basis for this application.

Laboratory-Based Disease Oriented Studies

Chronic Angiotensin II (AT1) receptor blockade improved renal function and enhanced renal cGMP production by endogenous natriuretic peptides and nitric oxide with in experimental overt CHF with cardiorenal syndrome[65]

A hallmark of overt/severe CHF is an attenuated renal cGMP production by endogenous natriuretic peptides (NP) and nitric oxide (NO), which may contribute to decline in renal function. Previous studies have demonstrated that Angiotensin II activate PDE V resulting in increased cGMP degradation. We therefore hypothesized that chronic AT1 receptor blockade would restore the renal cGMP by endogenous NPs and NO as assessed by urinary cGMP, with improved sodium excretion. We determined the cardiorenal actions of chronic AT1 blockade (Valsartan, Novartis, 320 mg daily for 10 days, n=5) in a canine model rapid ventricular

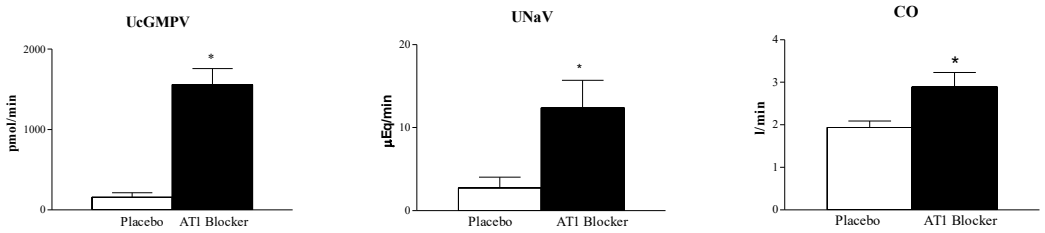


Figure 1. Urinary cGMP excretion, sodium excretion and cardiac-output. Open bar-placebo and shaded bar - AT1 blocker. * p<0.05

pacing induced overt CHF (245 bpm for 10 days) as compared to a non-treated group (n=5). After 10 days of chronic AT1 receptor antagonism, urinary sodium excretion increased (UNaV) (12.4±3.3 vs 2.7±1.3 uEq/min,p<0.05) in association with a marked increase in urinary cGMP excretion (UcGMPV) (1558±200 vs

139±65 pmol/min, p<0.05) as compared to the non-treated group. (Figure 1)The natriuretic response to AT1 receptor antagonism was localized to the inner medullary collecting duct, a nephron site rich in natriuretic peptide receptors, as distal tubular fractional sodium reabsorption decreased in the AT1 blocker group vs non-treated group (97.6±0.3 vs 98.9±0.5 %, p<0.05). These renal responses occurred in the absence of any changes in plasma NPs or cGMP. Chronic AT1 also resulted in marked decreases in cardiac filling pressures (RAP: 3.4±1.1 vs 10.4±1.6 mmHg, p<0.05 and PCWP: 15.4±1.9 vs 25.6±3.1 mmHg, p<0.05) and increased cardiac output (CO) (2.9±0.4 vs 1.9±0.2 l/min, p<0.05). (Figure 1) We conclude that chronic AT1 receptor antagonism in experimental overt CHF enhances the renal cGMP production, the common secondary messenger for the NPs and NO resulting in improved renal tubular function and sodium excretion. This study provides insight into renal and humoral pathophysiological actions of angiotensin II and the AT1 receptor in CHF and mechanisms by which AT1 receptor antagonism may mediate beneficial therapeutic properties.

Angiotensin II AT1 receptor blockade prevents the detrimental renal actions of acute diuretic therapy in human heart failure in compensated human CHF which are mediated via the renin-angiotensin aldosterone system [31]

To better understand the effects of renin-angiotensin aldosterone system (RAAS) on renal function in human heart failure, we defined the renal hemodynamic and humoral actions acute AT1 blockade (Losartan, 50 mg orally) vs placebo in combination with Furosemide in 10 NYHA II-III CHF patients in a double-blind placebo controlled cross-over study. Furosemide with placebo resulted in increased sodium excretion (212±66 to 425± 82 μEq/min, p<0.05) associated with reductions in both renal blood flow (307±30 to 246±23 ml/min, p<0.05) and glomerular filtration rate (76±8 to 63±6 ml/min, P<0.05). (Figure 3) However, after 4 hrs, there was a significant reduction in sodium excretion as compared to baseline (212±66 to 104±21 μEq/min, p<0.05). In contrast, Lasix with Losartan resulted in a similar increase in sodium excretion without reductions in renal blood flow and glomerular filtration rate. Furthermore, after 4 hrs, sodium excretion was greater as compared to the placebo group. Plasma aldosterone decreased only in the losartan group. This study suggests that the RAAS via AT1 receptors may be involved in the detrimental renal effects of diuretics in CHF. Therefore, antagonizing the RAAS in CHF patients on diuretics may improve renal function, which is an important predictor of survival in human CHF.

RESEARCH DESIGN AND METHODS

Objectives

The main objective of this study is to translate the findings of the applicant's study in experimental CHF to human CHF and determine the modulating actions of chronic AT1 receptor blockade in addition to ACE inhibitor, beta-blocker and furosemide on cardiorenal and humoral function in humans with compensated CHF and renal dysfunction. Preliminary studies completed by the applicant in experimental CHF have shown improvement in left ventricular function and enhanced renal function with AT1 receptor blockade. (see Preliminary studies) Furthermore, the applicant also completed a study in human compensated CHF demonstrating that acute AT1 blockade prevented the detrimental renal effects of furosemide therapy. (see Preliminary studies)

Hypotheses

1. Chronic AT1 receptor blockade in subjects with compensated CHF and renal dysfunction will improve renal function with increased sodium excretion, glomerular filtration rate and effective renal plasma flow as compared to the response of placebo-treated subjects.

2. Chronic AT1 receptor blockade in subjects with compensated CHF and renal dysfunction will improve humoral function with suppression of the aldosterone and increase renal cGMP generation as compared to placebo.

3. Chronic AT1 receptor blockade in subjects with compensated CHF and renal dysfunction will improve left ventricular systolic and diastolic function and filling pressures as compared to placebo as evident by:
    - Improvement in ejection fraction

- Decrease in filling pressures with a decrease in the ratio of early mitral diastolic Doppler flow velocity (E) to tissue Doppler velocity (e') of the mitral annulus (E/e')
- Improved indices of diastolic function with an increase in the e' velocity (faster relaxation)
- Improvement in clinical status as measured by 6-min walk and MLWHFQ

Study design

This will be a **double-blinded placebo controlled study design**. Subjects will be randomized to the Chronic AT1 receptor blockade or to the placebo group. We will compare the effect of 12 weeks of Chronic AT1 receptor blockade with Candesartan (n=38) or placebo (n=38) on cardiorenal and humoral in subjects with compensated CHF and renal dysfunction

Specific methods

i) <u>Study population</u>

76 Subjects with compensated CHF with renal dysfunction

<u>Inclusion Criteria:</u>

- Left ventricular ejection fraction of equal or less than 40% assessed by echocardiography, nuclear scan, MRI or left ventriculogram within the past 48 months.
- Stable New York Heart Association (NYHA) class II and III symptoms as defined by: no change in NYHA symptoms over the past 3 months, on stable doses of ACE inhibitor, beta blocker, digoxin and furosemide over the last 4 weeks and no episode of decompensated CHF over the past 6 months.
- Calculated creatinine clearance of less than 90 ml/min and greater than 20 ml/min, using the MDRD formula assessed within the past 48 months and a confirmatory calculated creatinine clearance equal or less than 80 ml/min and greater than 20 ml/min at the time of enrollment.

Subjects who are already taking AT1 receptor blocker will be excluded. Aldosterone antagonist, antiarrhythmic medications and other vasodilators will be allowed; however, all cardiac medications must be at stable doses 4 weeks prior to initial testing visit. Subjects taking nonsteroidal anti-inflammatory drugs (NSAIDs) except aspirin will not be able to increase their medication dose for the duration of the study. Subjects will be excluded if they have had a prior diagnosis of intrinsic renal disease, including renal artery stenosis of > 50%, or if they meet any one of the exclusion criteria listed below.

<u>Exclusion criteria specification</u>

- Prior diagnosis of intrinsic renal diseases including renal artery stenosis of > 50%
- Peritoneal or hemodialysis within 90 days or anticipation that dialysis or ultrafiltration of any form will be required during the study period
- Hospitalization for decompensated CHF during the past 6 months
- Subjects that are taking AT1 receptor blockers
- Myocardial infarction within 6 months of screening
- Unstable angina within 6 months of screening, or any evidence of myocardial ischemia
- Significant valvular stenosis, hypertrophic, restrictive or obstructive cardiomyopathy, constrictive pericarditis, primary pulmonary hypertension, or biopsy proven active myocarditis
- Severe congenital heart diseases
- Sustained ventricular tachycardia or ventricular fibrillation within 14 days of screening
- Second or third degree heart block without a permanent cardiac pacemaker
- Stroke within 3 months of screening, or other evidence of significantly compromised CNS perfusion
- ALT >1.5 times the upper limit of normal
- Serum sodium of < 125 mEq/dL or > 160 mEq/dL
- Serum potassium of < 3.5 mEq/dL or > 5.7 mEq/dL
- Serum digoxin level of > 2.0 ng/ml
- Hemoglobin < 9 gm/dl
- Other acute or chronic medical conditions or laboratory abnormality which may increase the risks associated with study participation or may interfere with interpretation of the data
- Received an investigational drug within 1 month prior to dosing

- Patients with an allergy to iodine.
- Female subject who is pregnant or breastfeeding
- In the opinion of the investigator, is unlikely to comply with the study protocol or is unsuitable for any reasons

ii) Study protocol

After enrollment into the study, diet instructions will be given by a dietician about a no added salt diet, 2-3 gm salt/day and 0.75 gm protein/Kg/day diet which will be maintained throughout the study period. Baseline chemistry, ALT and complete blood count will be obtained and subjects taking NSAIDs except for aspirin will not increase their dose until the end of the study. A urine pregnancy test will be performed, if necessary.

Twenty-four hour urine collection will be obtained one day prior to the active renal clearance study day for assessment of sodium excretion, creatinine clearance and microalbuminuria. . Sulfa containing medications and Probenecid will be withdrawn for 72 hours prior to the active renal clearance study days. Patients will be instructed to avoid strenuous physical activity and to abstain from smoking and alcohol-containing drinks three days prior to active renal clearance study day.

After the three-week stabilization period subjects will fast overnight and be admitted to the CRU on the morning of the active study day where they will undergo a 6 min walk. Their first urine void after admitting will be collected for protein analysis and urine pregnancy test (if necessary).. They will complete the Minnesota Living with Heart Failure Questionnaire (MLHFQ) during their stay. Their usual morning dose of medications (except diabetic medications) will be taken at 07:00 upon admission. The subjects will be placed in the supine position for 1 hour. During the first 15 minutes, two standard intravenous (IV) catheters will be placed (one in each arm). One catheter will be used for infusion and the other (in the contralateral arm) for blood sampling. Subjects will be asked to drink 5 mL/Kg of water initially and then drink the amount equal to the urine output every 30 minutes to ensure sufficient urinary flow. A priming dose of Iothalamate (0.0053 mL/Kg) to measure glomerular filtration rate (GFR) will be infused, followed by a constant rate IV sustaining dose (calculated according to estimated kidney function) of Iothalamate to achieve steady-state plasma concentrations of 15 to 20 mg/L. The subjects will be asked to empty their bladder spontaneously every thirty minutes.

After an equilibration period of 45 minutes, urine and blood samples are collected at 30-minute intervals for 3 periods to determine the baseline GFR and urinary sodium excretion (UNaV). Blood pressure will be measured at 20-minute intervals by using automatic blood pressure cuff, and heart rate will be continuously monitored. Urinary samples for determination of volume, sodium, potassium, Iothalmate, and PAH, will be obtained at the end of each clearance period. Venous blood samples for Iothalmate, PAH and sodium will be obtained at the middle of each clearance period. During the first clearance, venous blood samples for renin, aldosterone, angiotensin II, ACE, ACE 2, angiotensin 1-7, ANP, BNP, Bradykinin, NO, DNA, protein and cGMP will be obtained and urine samples for cGMP will also be obtained. BUN will also be run. Echocardiography will be performed after these baseline clearances as described.

At the completion of the baseline assessments, subjects will be randomized to Candesartan or placebo group. The randomization schedule will be provided by the Division of Biostatistics, implemented by the Mayo Pharmacy and administered by the CRU staff. The investigators and the CRU staff will not be aware of the randomization arm the subject is assigned to.

Subjects will receive the first dose of study drug in the CRU. Subjects will be started at 4 mg once a day. Thereafter, both blood pressure and heart rate (by electrocardiography) will continue to be monitored for the next 4 hours. If the patients develop symptomatic hypotension as defined by systolic blood pressure of =< 85 mmHg, with lightheadedness, dizziness or visual symptoms after the first dose of the medication in the CRU, the patient will be excluded from the rest of the study. The dose will be doubled every 14-18 days as tolerated to a goal of 16 mg a day or highest dose tolerated. Patients will get blood draw 7-10 days after each dose increase to measure plasma creatinine and potassium until target dose is achieved. I*f there should be a potassium result <3.5 or >6.0, subjects will need to have labs redrawn the same day.*Thereafter, subjects will get blood draws every 4 weeks (+/- 3 days). If distance indicates, blood draws will be arranged at the participants local clinic. Subjects will also have access to a 24-hour phone number should they have any questions or develop any side effects. They will be instructed to measure their blood pressure weekly and

record it, along with heart rate and weight. They will also receive a weekly phone call to review status. During the study, if patient develops symptomatic hypotension as defined above or an increase of plasma creatinine of > 0.3 mg/dL or hyperkalemia > 5.7 meq/ml, the dose will be reduced by 50%. If those parameters persist, the dose will be decreased further by 50%. At the discretion of the investigator, the medication can be discontinued.

At the end of the twelve-week study period (± 7 days), subjects will be admitted to the CRU again. A 6-min walk, MLHFQ, echocardiography, renal clearance, humoral determination and renal functional reserve will be performed in the same manner as the baseline study. Subjects will also perform a 24-hour urine collection the day prior to their return visit for determination of sodium excretion, creatinine clearance and microalbuminuria. Subjects will be dismissed after the renal clearance study.

iii) Sample size and statistical analysis

The sample size calculation and statistical analysis have been designed in collaboration with the Division of Biostatistics, (Kent Bailey, Ph.D and David Hodge, MSc).

The **primary analysis** for this study will be a comparison of the parameters of interest between the AT1 receptor blockade group and placebo. This analysis will be completed using a two-sample t-test comparing the change from baseline to the latest time point available. In addition to this primary analysis, if complete or near complete data is available, a secondary analysis will be completed using all data from baseline to the latest available time point. The groups will be compared in this analysis using a 2 factor repeated measures analysis of variance. Values will be expressed as the mean ± SE. A statistically significant difference will be considered to be present when $p<0.05$.

Sample size calculation: Preliminary data were obtained from the applicant's previous study. These data were used to estimate the standard deviation of the change in each parameter in the treated group. It was assumed that the standard deviation in the untreated group would be only ½ of that in the treated group, since the treated group is composed of responders and non-responders, while the untreated group response data is largely due to biological and measurement variability between 2 occasions, with little or no actual signal. In light of this argument, allocation will be unequal in order to optimize the precision of the group comparison. Specifically, there will be 2 treated subjects per 1 placebo subject. Preliminary data for this analysis is provided below.

	Change from Baseline in AT1 group	Change from Baseline in Placebo group	N
GFR ml/min	-20.67±15.97	1.13±12.18	6
Na excretion mEq/min	-11.89±27.27	-10.78±20.08	6
ANG II pg/ml	29.32±107	11.98±43.67	6
Renin ng/ml/hr	4.9±6.5	3.9±4.4	6

So based on this preliminary data, the study will have 90% power to detect the following differences using 38 patients in the AT1 receptor blocker group, and 38 in the placebo group.

	Detectable change	N/group
GFR ml/min	15	38 and 38
Na excretion mEq/min	25.4	38 and 38
ANG II pg/ml	100	38 and 38
Reninng/ml/hr	6.1	38 and 38

Data Handling

The study coordinator will enter data into a Microsoft Excel spreadsheet. In addition to cardiorenal and humoral data mentioned above, 6 min walk, MLWHFQ scores and clinical data including cardiovascular risk factors, medications, clinical events, laboratory data and physical exam will be collected. Data will be stored on a

secure site on the Mayo Clinic computer server to ensure all safety protocols established by the clinic are in place. This will also provide adequate backup of the data. Printouts of the computer information will be made and stored in the cardiovascular office for data backup. At the conclusion of the study, Dr Kent Bailey and Mr. David Hodge, Division of Biostatistics using methods as described above, will analyze the data. Analysis will be completed using SAS Proprietary Software Release 8.2 (1999-2000 SAS Institute, Inc., Cary, NC). Guidelines established by the Department for the handling and storage of research data will be followed. Standardized systems have been developed to ensure that patient confidentiality is maintained. Each member of the department has undergone training in these systems at the time of their initial and ongoing employment training. They include username/password access to data and programs; the ability to log access; patient data displayed by numeric id not patient name; the use of numeric identifications on all patient listings and summary reports of statistical analyses. All data sets and computer programs are logged within the Division of Biostatistics with respect to Institutional Review Board authorization, principal and secondary investigators, lead statistician, team statisticians, and accounting information. Working data are maintained on a single large file server (over 350 GB of user space) which serves the entire Section. Inactive data files are moved to archival storage under the control of an automated system, itself controlled by a DBMS based request system which ensures that all data movement is appropriately logged and commented. The archival storage is hosted on the institutional mainframe computer. The use of the mainframe ensures several high-level support functions for the archive system, e.g. storage of media in separate fire zones and regular copying of data to new media. All active data and programs are maintained on a central password-protected (UNIX) system accessible to the members of the Division of Biostatistics. An audit trail of file access is available if necessary. Active data files are backed-up nightly and are available for retrieval at any time.

Recruitment of subjects

Subjects meeting the inclusion criteria of the study will be recruited at the Mayo Clinic from the Heart Failure Clinic, Community Internal Medicine, General Internal Medicine, and past participants of Dr. Chen's studies. The **Mayo Clinic Life Science System** (MCLSS) will aid recruitment for all the protocols. [redacted] Currently we have approximately 80 patients who would qualify for Specific Aims 1 and 2. Hence using a conservative estimate of 20% recruitment success, we project a recruitment of 16 patients for Aims 1 and 2 in 6 months, hence we will complete Specific Aims 1 and 2 in the 5 years as stated below.

Recruitment will be limited to men and women aged 18 years and above. Female participants must be postmenopausal or have been surgically sterilized or have a negative pregnancy test and must agree to use a reliable double barrier method of contraception until study completion. A double barrier method of contraception is considered to be a combination of TWO of the following: birth control pill/implants/injections, intrauterine devices, spermicide, diaphragm or condoms.

Upon identification of a subject, the study will be explained in detail, the consent form reviewed, questions answered and the consent form signed. Protocol procedures will then begin to be scheduled. Subjects will receive a reimbursement of $400 for participation in the study.

Assessment of systolic and diastolic function by echocardiography

Transthoracic echocardiography will be performed by the certified sonographer of the Mayo Clinic Echocardiography Laboratory. The Principal investigator, Horng Chen MD, who has level III echocardiography certification, will review all studies in a blinded fashion. Echocardiographic images will be obtained from standard acoustic windows according to the recommendations of the American Society of Echocardiography.
Assessment of pulmonary artery pressure: In the subcostal window, imaging of the inferior vena cava and hepatic veins will be performed and used to estimate RA diastolic pressures. Pulmonary artery pressure will be calculated from the tricuspid regurgitation velocity and the estimated RA diastolic pressure.
Ventricular structure: Ventricular volumes (Systolic and Diastolic) will be assessed by Biplane Simpsons (method of discs) analysis of LV volume obtained from two orthogonal cuts of the LV (apical 4 and 2 chamber).
Systolic function. Ejection fraction (EF) will be calculated from the M-mode measurements and from the volumes as calculated from the Biplane Simpsons analysis using the formula: EF (%) = 100 * (Diastolic Volume – Systolic Volume)/ Diastolic Volume
Assessment of LV diastolic function and LV filling pressures. Pulsed wave Doppler examination of mitral (before and with Valsalva maneuver) and pulmonary venous inflow as well as Doppler tissue imaging of the mitral annulus will be performed.

Analytic methods
Both plasma and urine concentration of Iothalmate will be determined by the Mayo Core Renal lab. Glomerular filtration rate (GFR) will be calculated using Iothalmate clearance using the following equation:

$$GFR\ (ml / min) = \frac{U \times V}{P}$$

[Where: U = urine concentration; P = plasma concentration; V = urine flow (mL/ min)]
Plasma and urine will be analyzed for sodium with the Beckman ion-selective analyzer. Urine creatinine will be measured by the Mayo Core Renal Laboratory. Renal cGMP generation, will calculated as follows: {[urine cGMP * urine flow] – [plasma cGMP* GFR]}. Venous blood for hormone analysis will be collected in heparin and EDTA tubes and immediately placed on ice. After centrifugation at 2,500 rpm at 4C, the plasma will be decanted and stored at -80C until analysis. Urine for cGMP radioimmunoassays will also be collected on ice as previously described.[31,67] Specific radioimmunoassays include renin, aldosterone, angiotensin II, angiotensin 1-7 ANP, BNP, Bradykinin, NO and cGMP will be carried out in the co-investigator Dr Burnett's laboratory, utilizing well established methodologies in the laboratory.[68] ACE and ACE 2 will be measured by high-performance liquid chromatography with electrochemical detection.

Potential risk and protection against risk

E. Human Subjects Research

This Human Subjects Research meets the definition of a clinical trial.

Human subject involvement and characteristic
The specific subject population is described in the Specific Methods for each protocol. The inclusion and exclusion criteria are listed in the Specific Methods. These protocols will not involve special classes of subjects, such as fetus, neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered vulnerable populations.

Source of materials
Plasma samples, urine samples echocardiographic data and clinical data will be used entirely for research purposes. Patient identifiers will not be released outside of Mayo and any publications will exclude any kind of patient identifiers that could be correlated with the specific patient.

Potential risks and Protection against risk

The overall risk for participation in this protocol is small and the main concern is that the addition of Candesartan (AT 1 blocker) and or increasing the ACE inhibitor dose would result in hypotension, hyperkalemia, and increased plasma creatinine. However, we demonstrated that acute AT1 receptor blockade prevented the detrimental renal hemodynamic effects of furosemide with suppression of aldosterone. Previous studies have also established beneficial renal hemodynamic and excretory function of AT1 receptor blockade in experimental models of CHF. Vople et al demonstrated that 6 weeks of AT1 blockade improve renal functional reserve in patients with mild CHF. Furthmore, the Val-HeFT and Charm studies have demonstrated that AT1 blockage can be added on safely in patient with stable CHF.

To protect the patients against the risk of hypotension, hyperkalemia, and increased plasma creatinine, the following measure are incorporated in the protocol:

- We will only include patients with compensated stable CHF as defined by; a) no change in NYHA symptoms over the past 3 months; b) on stable doses of ACE inhibitor, beta blocker, digoxin and furosemide over the past 4 weeks; c) no episode of decompensated CHF over the past 6 months. Theis will minimize their risk of fluid retention and congestion.
- We will exclude patients with prior diagnosis of intrinsic renal diseases including renal artery stenosis of > 50%
- We will exclude patients with Serum potassium of > 5.7 mEq/dL
- The protocol for Specific Aims 2 will be initiated in the CRU under close monitoring. Subjects will receive their first dose of study drug in the CRU. Thereafter, both blood pressure and heart rate will continue to be monitored for the next 4 hours. If the patients develop symptomatic hypotension as defined by systolic blood pressure of =< 85 mmHg, with lightheadedness, dizziness or visual symptoms after the first dose of the medication in the CRU, the patient will be excluded form the rest of the study.
- Patients will have blood drawn to measure plasma creatinine and potassium 7-10 days after every dose increase until target dose is achieved. Thereafter, subjects will get blood draws every 4 weeks. I*f there should be a potassium result <3.5 or >6.0, subjects will need to have labs redrawn the same day.*
- Subjects will also have access to a 24-hour phone number should they have any questions or develop any side effects.
- They will be instructed to measure their blood pressure weekly and record it, along with heart rate and weight.
- They will also receive a weekly phone call to review status.
- During the study, if patient develops symptomatic hypotension as defined above or an increased of plasma creatinine of > 0.3 mg/dL or hyperkalemia > 5.7 meq/ml, the dose will be reduced by 50%. If those parameters persist, the dose will be decreased further by 50%. At the discretion of the investigator, the medication can be discontinued

The following stopping rule will be applied to ensure patient safety

If the patients develop symptomatic hypotension as defined by systolic blood pressure of =< 85 mmHg, with lightheadedness, dizziness or visual symptoms after the first dose of the medication in the CRU, the patient will be excluded from the rest of the study. During the study, if patient develops symptomatic hypotension as defined above or an increase in plasma creatinine of > 0.3 mg/dL or hyperkalemia > 5.7 meq/ml, the dose will be reduced by 50%. If those parameters persist, the dose will be decreased further by 50%. If those parameters persist, the subject will be withdrawn from the study

Other potential minor risk includes:

The risks of blood drawing include bleeding at the puncture site, bruising and pain. These risks occur in a very small portion of the population. We will exclude patients with hemoglobin of < 9 g/dl. The blood draw amount for this study is 200 mLs.

This protocol may be hazardous to an unborn child. There is no medical information to determine whether there are significant risks to a fetus carried by a mother who is participating in this study. Therefore, female

participants must be postmenopausal or have been surgically sterilized or have a negative pregnancy test and must agree to use a reliable double barrier method of contraception until study completion. A double barrier method of contraception is considered to be a combination of TWO of the following: birth control pill/implants/injections, intrauterine devices, spermicide, diaphragm or condoms.

Iothalamate contains a small amount of iodine and for persons allergic to iodine, this could pose a higher risk for an allergic reaction. To date in more than 5,000 patients, there have been only rare allergic reactions (one or two) when the solution has been used for this type of test. The amount of the material injected for this test will be approximately the same as used in a similar kidney function test performed on a routine basis (Approximately 250 test per month) in the Renal Function Laboratory. However, patients with a history of an allergic reaction to iodine should not participate in this study.

Adequacy of protection against risks

Recruitment and informed consent
Subjects will be recruited at the Mayo Clinic from the Heart Failure Clinic, Community Internal Medicine, General Internal Medicine, and past participants of Dr. Chen's studies. In addition, we also now have in place a new web-based patient identification system developed at Mayo by the Cardiovascular Research Program. Upon identification of a subject, the study will be explained in detail by the investigators or study co-ordinator, the consent form reviewed, questions answered, and the consent form signed.

Potential benefits of the proposed research to the subjects and others
Subjects participating in specific aims 2 may potentially benefit from this study, with improved ventricular, renal and humoral function. More importantly, these studies will provide us new insights into the human cardiorenal syndrome that may allow us to develop new therapeutic strategies, which may benefit others with the same problem.

Importance of knowledge to be gained
This research will provide us new insights into the human cardiorenal syndrome that may allow us to develop new therapeutic strategies, which may benefit others with the same problem.

Data and safety monitoring plan

All clinical trial protocols carried out in the Mayo Clinic will have a Data Safety and Monitoring Plan incorporated into the protocol which will have to be reviewed and approved by Mayo Institutional Review Board (IRB). Our plan includes the formation of an internal safety monitoring committee consisting of 2 cardiologists: Dr [redacted] and Dr [redacted], and one nephrologist, Dr. [redacted]. All adverse and serious adverse events occurring between consent and final study visit will be reported to the safety monitoring committee, NIH and our IRB. Furthermore, the safety monitoring committee will review adverse events after 10 and 30 subjects have completed the protocol. The recommendations of the safety committee after each review will be submitted to the IRB. All serious adverse events will be reported as described below.

A plan for reporting Serious Adverse Events (SAE) to the IRB

A **Serious Adverse Event** is defined as an event with any of the following outcomes:

- Death
- Life-threatening adverse drug or device experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

Unanticipated Problem Involving Risk to Subjects or Others (UPIRTSO):

Mayo Clinic's IRB and OHRP defines unanticipated problem/event involving risk to subjects or others (UPIRTSO) as any problem or event that was 1) serious; 2) unanticipated; AND 3) at least possibly related to the research procedures.

Serious: Serious problems/events can be well defined and include death; life threatening adverse experience; hospitalization: inpatient, new, or prolonged; disability/incapacity: persistent or significant; birth defect/anomaly; and/or per protocol OR may be problems/events that in the opinion of the local Investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

Unanticipated: (unexpected) problems/events are those that are not already described as potential risks in the consent form, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem/event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem/event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected.

Related: A problem/event is "related" if it is possibly, probably or definitely related to the research study

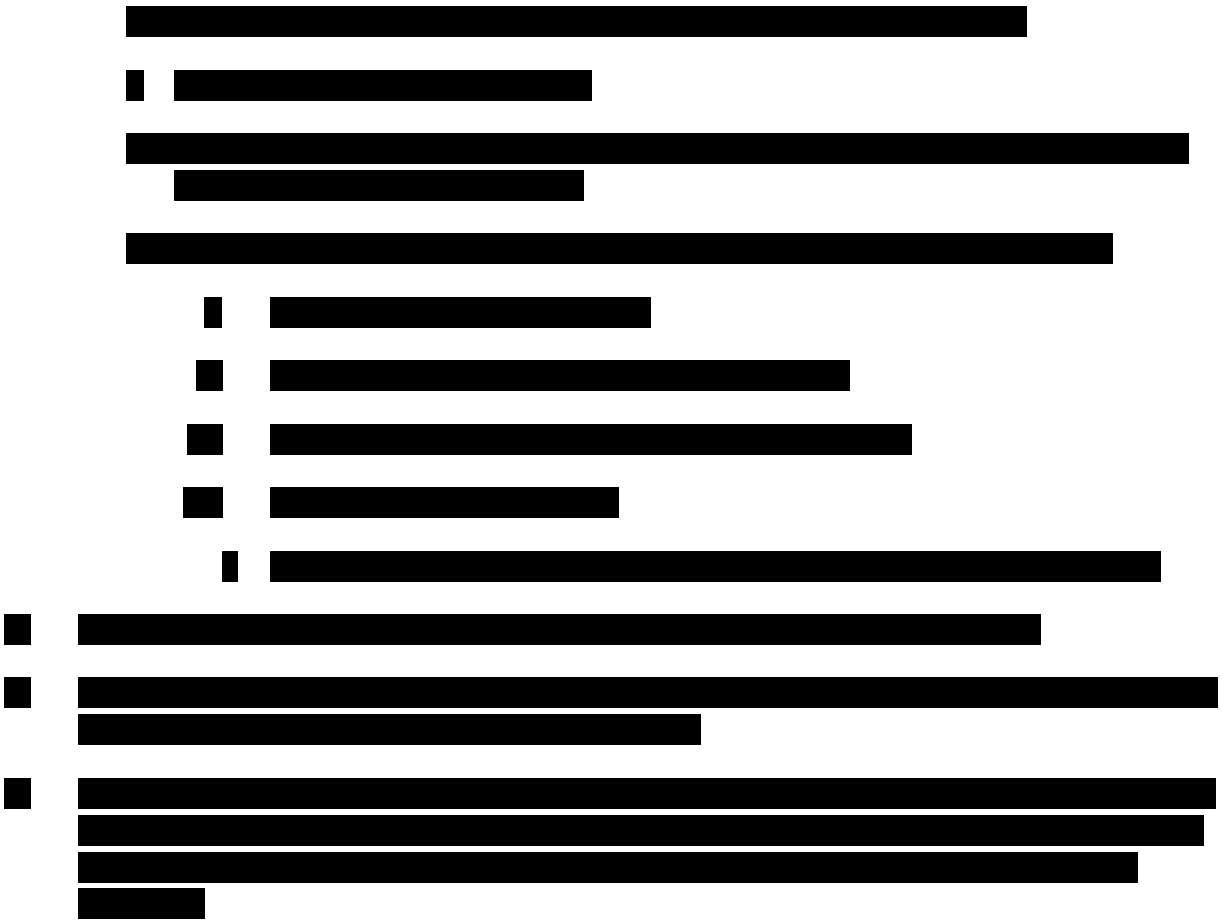
Mayo IRB

Regardless of causality, the investigator will report SAE to DSMB within 72 hours of knowledge of the event to determine the relationship between the event and the study intervention. If the event was definitely related, probably related or possibly related to the study intervention, the PI will submit the UPIRTSO form to Mayo IRB within 72 hours, and include any relevant documents such as medical record notations or reports with the name and medical record number of the individual removed. Assess whether protocol modifications are required as a result of the UPIRTSO. The Investigator must provide an explanation on the UPIRTSO form explaining what corrective actions have already been taken.

The following section is the Mayo IRB's policy for handing a UPIRTSO report:

[redacted]

[redacted]



3. The names and contact information for study personnel to be contacted in the event of questions regarding adverse events and serious adverse events among study participants.

Subjects can call Dr. Horng Chen, telephone [redacted], at any time about any question on the studies including adverse events and serious adverse events.

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