Effect of the renal natriuretic peptide, ularitide, alone or combined with Vasopeptidase inhibitor, Omapatrilat, on experimental volume overload-induced congestive heart failure in rats (Ularitide/Omapatrilat in Congestive Heart Failure)

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KEYWORDS
Congestive heart failure; Aorto-caval fistula; ularitide; Omapatrilat; Renin-angiotensin system; Vasopeptidase inhibition

Abstract Introduction: Ularitide is a synthetic form of renally derived natriuretic peptide (NP), urodilatin. Omapatrilat (OMA) is a Vasopeptidase inhibitor (VPI), acting by dual inhibition of both angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP), which degrades the NPs. Ularitide and OMA underwent evaluation for the management of hypertension and heart failure (HF).

Aim: This study aimed to address the effect of ularitide and OMA in aortocaval fistula (ACF) – induced congestive heart failure (CHF) in rats under various conditions of compensation (of clinical severity).

Experimental protocol: Volume-overload CHF was induced in male albino rats by creating an infrarenal ACF. One week after fistula induction, ACF rats were randomized to compensated (Com) and decompensated (Decom) ACF groups and each further subdivided into ACF, ularitide and OMA/ularitide treated ACF groups. Sham was used as control. All treatment protocols were started one week after infrarenal ACF induction and continued for further two weeks. Three weeks after shunt induction, all animals were underwent assessment of cardiorenal and humoral functions. Renal outcome was measured by glomerular filtration rate (GFR), fractional excretion of sodium ($E_{Na}$), absolute urinary sodium excretion ($UNaV$), urine volume, plasma cystin C level and urinary cyclic 3', 5'-guanosine monophosphate (cGMP). The humoral function was assessed by plasma renin activity (PRA), angiotensin II (Ang II), Aldosterone, and cGMP. Cardiac outcome was assessed by plasma atrial natriuretic peptide (ANP), N-terminal pro–brain natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin T (hs-cTnT) while total and relative heart, lung and liver weights were recorded.

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1. Introduction

Congestive heart failure (CHF) is a major, expanding public health problem with a poor prognosis and the most common cause for hospitalization of elderly patients. CHF is a syndrome characterized by myocardial structural and functional impairment and a severe disturbance of body fluid balance. The immediate clinical goal in managing HF is to provide symptom relief and to stabilize the patients' hemodynamics. Unfortunately, these treatments often only provide symptomatic relief and temporarily increased risk of renal deterioration and impede disease progression. Thus; the search for agents that improve HF signs and symptoms and preserve renal function without increasing mortality risk has been an area of ongoing research.

Volume overload represents a clinically relevant condition leading to HF, for example in aortic or mitral valve insufficiency. The rat model of chronic HF due to volume overload induced by aortocaval fistula (ACF) is simple, reproducible and well-characterized model of CHF, sharing many similarities with the natural course of human HF. This artificial AC shunt causes a marked increase in venous return (VR) and cardiac output (CO) that triggers compensatory, initially asymptomatic cardiac hypertrophy. With persistent hemodynamic overload and redistribution of CO, it leads to gradual transition from asymptomatic into the decompensated phase, marked renal and neurohumoral changes that closely mimic those found in patients with advanced HF. These include reduced renal blood flow and GFR, urinary salt and water retention, activation of the RAAS, and increased secretion of, but blunted response to, ANP. Ularitide (urodilatin) is a natriuretic peptide (NP) composed of 32 amino acid residues [ANP-(95–126)] with a primary structure similar to ANP (99–126) and it was isolated from human urine. Similar to ANP, ularitide binds to the natriuretic peptide-A receptor (NPR-A), with similar affinities, and is cleared by the natriuretic peptide-C receptor (NPR-C) and degraded by the ectoenzyme neutral endopeptidase 24.11 (NEP). ularitide is more active than ANP as a natriuretic agent and more resistant than ANP to degradation by NEP, as it exhibits an N-terminal extension by four amino acids compared with ANP. After synthesis in the distal tubular cells, urodilatin is luminaly secreted and binds further downstream in the inner medullary collecting duct to NPR-A, mediating its effects via increasing intracellular cyclic 3',5'-guanosine monophosphate (cGMP) levels.

Urodilatin administered intravenously (IV) to rats, dogs or healthy volunteers exhibits hemodynamic as well as diuretic and natriuretic effects. Omapatrilat (OMA), is a potent oral, long-acting dual metalloprotease inhibitor, the most extensively studied, to date. OMA has selective and competitive inhibitory activity of both angiotensin-converting enzyme (ACE) with subsequent suppression of RAAS and (NEP), which degrades natriuretic and vasodilator peptides such as adrenomedullin and bradykinin, heightening activity of these endogenous vasodilator systems leading to more potent hemodynamic and renal effects and improving myocardial function than the selective inhibition of the same enzymes.

The present study was carried out to evaluate the effect of ularitide and OMA in albino rats with the (ACF) model of high-output HF and exploring the differential response in compensated versus decompensated stage and the possible underlying mechanisms.

2. Materials and methods

2.1. Experimental animals

The experimental protocols were approved by the Ethical Committee for Animal Experimentation at the Faculty of Medicine, Tanta University and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male albino rats (250–300 g) were housed individually in metabolic cages under standard conditions with free access to standard rat diet and drinking water ad libitum throughout the study. They were acclimatized for one week prior to the experiment, and all efforts were made to minimize suffering.

2.2. Experimental CHF model: induction of ACF in rats

Under 40 mg/kg, of IP pentobarbital sodium anesthesia and complete septic conditions, CHF was induced by volume overload achieved by creation of the infra renal ACF, using a needle technique (18-gauge needle, diameter 1.2 mm) as originally described by García and Diebold, 1990, and employed and validated by many investigators.

Results: Induction of AC shunt was associated with deteriorated renal and excretory functions, activation of renin angiotensin aldosterone system (RAAS), elevated ANP with renal resistance to ANP, (NT-proBNP) and (cTnT), pulmonary and systemic congestion and marked cardiac hypertrophy. These changes were exacerbated in Decom-ACF. Ularitide treatment of ACF rats was associated with natriuresis, diuresis, enhanced GFR with RAAS inhibition. This effect was evident in Com-ACF, maximized by OMA but attenuated in Decom-ACF, restored by OMA treatment. Ularitide/OMA treatment had antihypertrophic, decongestant effect with preserved renal function, resulted in a marked improvement of animals' survival.

Conclusion: OMA potentiates the cardiorenal actions of ularitide in ACF-induced Com CHF and restoring its effect in Decom ACF, by simultaneously inhibiting ACE and NEP. OMA and ularitide could provide an effective therapeutic strategy for CHF.
Briefly, laparotomy was performed. The intestines were displaced laterally and wrapped with normal saline-soaked sterilized gauze to retain moisture. The aorta and vena cava between the levels of renal arteries and iliac bifurcation were then exposed by blunt dissection of the overlying adventitia. A 2-cm section of the vessels distal to the origin of the renal arteries was temporarily occluded (~30 s) with a pair of mini-clamps. At the midpoint between the two clamps, a 18-gauge needle (Braun Melsungen, Melsungen, Germany) held on a plastic syringe was inserted into the exposed abdominal aorta and advanced into the vena cava through the common wall between the vessels to create the shunt and manipulated from side to side to widen the opening between the aorta and vena cava. The needle was withdrawn. The aortic puncture site was immediately sealed with a drop of cyanoacrylate glue (Histoacryl, B. Braun GmbH, Tuttingen, Germany), and the clamp was removed 30 s later. Creation of a successful fistula was confirmed visually by the pulsatile bright flow of oxygenated blood into and swelling of the vena cava. The intestines were repositioned and the abdominal cavity was closed by absorbable suture. Post-surgical analgesia was provided by metamizole (dipyrone, 40 mg/kg s.c.). Sham-operated rats served as controls, underwent similar surgical procedures but without puncture of the vessels or AC Creation. After the surgical procedure was completed (~10 min), the rats were given 0.2 ml of 40,000 U/ml penicillin and allowed to recover and surgical analgesia was provided by metamizole (dipyrone, 40 mg/kg s.c.).

Seven days after the operation, the ACF rats were further subdivided, on the basis of their daily (UnaV), into rats with compensated (Com) CHF (moderate CHF, (UnaV) > 1200 μEq/24 h) and sodium retaining rats with decompensated (Decom) CHF (severe CHF, (UnaV) < 100 μEq/24 h). Rats with UnaV greater than 100 μEq/24 h and less than 1200 μEq/24 h were not included in this study.

2.3. Experimental design and treatment protocols

One week after the infrarenal AC anastomosis, the surviving rats from the ACF model and sham-operated groups were randomly assigned, according to daily (UnaV), to one of two treatment protocols, Com or Decom HF, each started one week after ACF and continued for additional two weeks, during which daily urinary volume and (UnaV) were obtained.

2.3.1. The first approach (Decom-HF protocol): the treatment of acute Decom HF

This experimental protocol included the rats (n = 45) that developed severe Decom HF and avid Na retention and experienced many of the symptoms commonly associated with CHF, such as dyspnea, lethargy, pallor in the extremities, and a reduction in appetite. These rats were subjected to ularitide or OMA/ularitide treatments that continued for 14 days. The rats were randomly allocated into the following groups: Sham operated control group: received isotonic saline solution, IP as a vehicle. Decompensated (Decom-ACF) group: received the same volume of isotonic saline solution, IP. ularitide treated Decom-ACF group: received ularitide (INN: synthetic URO, CardioPep Pharma GmbH, Hannover, Germany, supplied as lyophilized powder, was dissolved in isotonic saline solution), in a dose of 12 μg/kg/d, IP. OMA/ularitide treated Decom-ACF group: received combined ularitide (12 μg/kg/d, IP) and OMA (1 μmol/kg/d, IP). The OMA dose would result in maximal ACE and NEP inhibition. OMA was purchased from American Custom Chemicals Corporation (Cat#167305-00-2, San Diego, CA, USA).

2.3.2. The second approach (Com-HF protocol): the treatment of mild Com HF

The Com HF rats (n = 45), which eventually returned to sodium balance (UnaV > 1200 μEq/d) were included in this treatment protocol for 14 days and randomly assigned to the following groups: Sham-operated control group: received isotonic saline solution, IP. Com-ACF group: received the same volume of isotonic saline, IP. ularitide treated Com ACF group: received ularitide in a dose of 12 μg/kg/d, IP. OMA/ularitide treated Com ACF group: received combined ularitide (12 μg/kg/d, IP) and OMA (1 μmol/kg/d, IP).

2.4. Sampling

At the end of the treatment protocols, the animals were anesthetized deeply by sodium pentobarbital (70 mg/kg, IP) and killed by decapitation. Their chest was opened, and the blood was obtained directly from the heart. Blood samples were collected on ice, then divided and drawn into heparin and EDTA tubes, centrifuged for 15 min at 3500 rpm and kept frozen at −20 °C until assayed all at once. Plasma obtained from the EDTA-containing blood tubes was stored until assay of plasma ANP, cGMP and plasma renin activity (PRA), angiotensin II (AngII) and aldosterone, while the heparinized plasma from the second tube was used for plasma Na and creatinine assay.

Urine was collected on ice. Urine samples for cGMP analysis were heated to >90 °C before storage. All samples from each animal were assayed together to avoid inter assay variability. Urine samples which are centrifuged for 10 min at 7000 rpm then refrigerated until assayed for Na and creatinine.

2.5. Tissue weights

After decapitation, the heart, lung and liver were harvested instantaneously, placed on absorbent paper to remove excess blood, and wet weights were recorded. Wet to body weight ratios for these tissues were then calculated to assess the degree of the hypertrophy and congestion.

2.6. Renal parameters

24 h urine samples were collected from all groups throughout the experimental period to determine urine volume/24 h. Plasma and urine Na+ concentrations were assessed by flame photometry (Model FP 20 seac, Seac Radim Company, Italy) with chemical kits (BioMérieux, France).

2.7. Hormonal and biomarkers assay

Plasma ANP, (PRA), Ang II, and aldosterone, were measured by radioimmunoassay. Plasma and urine cGMP levels were measured using an immunoradiometric assay kit supplied
by IBL GmbH (Cat. #29071/75, Flughafenstrasse, Hamburg, Germany), according to the manufacturer’s protocol.

Plasma high-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro–brain natriuretic peptide (NT-pro-BNP), as sensitive and specific markers of myocyte injury and stress respectively, were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions. Plasma cystin C concentrations, as a marker of kidney injury, were measured by using ELISA kit (R&D Systems, America).

2.8. Analytical methods

Urine flow rate (UFR) was calculated by the following formula: $UFR = \frac{UV}{T \times BW}$, where $UV$ is the urine volume, $T$ is the time and $BW$ is the body weight of the rat. Fractional excretion of sodium (FENa) was calculated by $\frac{CNa}{GFR}$, where $CNa$ is the clearance of sodium. Clearance was calculated using the usual formula: $C = \frac{Ux \times UFR}{Px}$, where, $Cx$ is the clearance of substance x and $Px$ is the plasma concentration of x. Glomerular filtration rate (GFR) was measured as creatinine clearance ($CL_{CR}$, ml/min) according to the method described by Cameron and Gerger. $CL_{CR}$ was estimated from SCr using the Cockroft–Gault equation. SCr was measured by creatinine kit (Diamond diagnostic, Egypt) based on the Jaffé reaction.

Renal cGMP production was calculated as follows: renal cGMP generation = (urinary flow $\times$ urinary cGMP concentration) $\div$ (GFR $\times$ plasma cGMP concentration).

2.9. Rat survival status

During the experimental periods, cages were inspected for dead animals daily. The survival status and time of death of the rats in each group were recorded for generation of the survival curves. The carcass was weighed and a gross postmortem examination was performed. Postmortem examination revealed that decompensated rats developed additional signs of CHF, i.e. ascites, enlarged liver, lung congestion, and pleural effusions whereas these signs were mild or nonexistent in compensated rats.

3. Statistical analysis

Survival rate was analyzed by the Kaplan–Meier analysis using the log-rank test with construction of survival curve. One-way Analysis of variance (ANOVA) for repeated measurements was performed followed by Bonferroni test for analysis of data. All statistical analyses were performed using GraphPad Prism software release 5.0 (Graph Pad Software, San Diego, California, USA). A $p$ value less than 0.05 was considered statistically significant.

4. Results

4.1. Daily sodium excretion patterns in ACF and sham-operated controls rats (Fig. 1)

Before surgery, the daily $U_{Na}$ ranged between 1378 and 1843 μEq/24 h (mean = 1593) (on the basis of measurements in 120 rats during 6 consecutive days before the operation). All groups displayed an immediate postoperative decrement in sodium excretion, most likely related to the anesthesia and surgical stress. However, whereas in sham-operated rats the decrease in $U_{Na}$ lasted for 24 h and then returned to the presurgical base-line levels on the 2nd postoperative day. Two distinctly different patterns of sodium excretion were evident in ACF rats. Approximately 43% of the animals displayed progressive sodium retention, as depicted by the gradual decrease in $U_{Na}$. These animals did not show symptoms indicative of HF despite the presence of a patent ACF and the finding of a hypertrophied heart when these rats were killed.

Thus, on the basis of the above findings, it was possible to divide the ACF rats into two subgroups: animals that retained sodium and developed manifestations simulating severe (acute) decompensated HF and animals that compensated and

![Figure 1](image-url) The daily urinary sodium ($U_{Na}$) excretion patterns in ACF rats and in sham-operated controls. Two distinct patterns of $U_{Na}$ excretion in ACF rats. Values are mean $\pm$ SD. * $p < 0.05$ vs. Sham. Com; compensated, Decom; decompensated, ACF; Aorto-caval fistula.
returned to normal sodium balance simulating the mild controlled chronic HF in human.

4.2. Effect of ularitide/OMA treatment on humoral profile in ACF rats (Fig. 2)

The activity of RAAS is enhanced in correlation with the severity of the cardiac dysfunction and provides a prognostic index in most HF patients. Three weeks after shunt induction, all ACF rats displayed significant activation of renin angiotensin system with secondary hyperaldosteronism. The PRA increased about 1.1 fold in Com-ACF compared to 2.5 fold increased in Decom-ACF versus sham animals. The increased PRA resulted in increased plasma Ang II level by 1.3 fold in Com-ACF compared to 2.6 fold in Decom-ACF versus sham and increased plasma aldosterone by about 1.1 fold in Com-ACF compared to 2.9 fold increase in Decom-ACF versus sham control group.

It appears that the systemic RAAS activation is most pronounced in acute Decom HF.

Ularitide treatment to Com-ACF rats resulted in significant suppression in RAAS, an effect that is amplified by concomitant OMA treatment to level comparable to that of sham group. While ularitide treatment to Decom-ACF rats resulted in decreased PRA and Ang II levels compared to Decom-ACF untreated rats.

Figure 2  Effect of Ularitide/OMA treatment on humoral profile and biomarkers of cardiac stress and injury: plasma renin activity (A), Ang II (B), aldosterone (C) and biomarkers of cardiac stress (ANP (D), NT-proBNP (E)) and injury (cTnT (F)) in ACF rats. Values are mean ± SD. *p < 0.05 vs. Sham, †p < 0.05 vs. Com-ACF, ‡p < 0.05 vs. Ularitide treated Com-ACF, ¶p < 0.05 vs. De-ACF, §p < 0.05 vs. Ularitide treated Decom-ACF. Ang II; angiotensin II, ANP; atrial natriuretic peptide, Com; compensated, Decom; decompensated, ACF; Aorto-caval fistula, cTnT; High-Sensitivity Troponin T, NT-proBNP; N-terminal of the prohormone brain natriuretic peptide OMA; omapatrilat.
in slight reduction in RAAS activity, combined OMA and ularitide treatment resulted in dramatic suppression of RAAS in the Decom group.

4.3. Effect of ularitide and OMA on biomarkers of renal (Table 2, Fig. 3) and cardiac injury and stress (Fig. 2)

Both the troponins and the NPs are gold standard biomarker classes that share the unique feature of being secreted directly, and almost exclusively, by cardiac tissue. Thus, they were proposed as a diagnostic tool in the determination of the cardiac dysfunctions and as a prognostic marker in the prediction of the survival of the HF patients, even, more sensitive than that obtained from imaging or even invasive hemodynamics.6

All ACF animals displayed high circulating levels of ANP compared to sham, reaching 3.4 fold in Com-ACF versus 6 fold increase in Decom-ACF rats. In spite of the dramatic increase in ANP in the Decom group, the rats still retained sodium.

With HF induction, the Com rats displayed a significant increase in the indicator of the ventricular wall stress, NT-proBNP 2.2 and 7 fold respectively in Com and Decom-ACF rats respectively versus sham control group.

Ularitide treatment to ACF rats resulted in significant reduction in ANP and NT-proBNP, an effect that is amplified by concomitant OMA treatment to levels comparable to that of sham group in Com.

In spite the Com-ACF animals had cTnT level comparable to that of the sham group, a significant myocardial cell injury

**Figure 3** Effect of ularitide/OMA treatment on plasma cyst –C (compensated (A) and decompensated (B), plasma and urinary CGMP (compensated (C, E) and decompensated (D, F) ACF rats. The mean (horizontal line) and individual values are shown in each experimental group. *p < 0.05 vs. Sham. †p < 0.05 vs. Com-ACF. ‡p < 0.05 vs. Ularitide treated Com-ACF. $p < 0.05 vs. De-ACF. $p < 0.05 vs. Ularitide treated Decom-ACF. Com; compensated, Decom; decompensated, ACF; Aorto-caval fistula, OMA; omapatrilat, CGMP; cyclic guanosine monophosphate, cyst –C; cystin C.
was detected in Decom-ACF group as indicated by cTnT leak into serum with significant elevation compared to the sham group.

The ularitide treatment had an obvious cardio protective effect which is marked by significant decrease in cTnT level and augmented in OMA/ularitide compared to Decom-ACF group.

Cystin C as an endogenous marker of GFR, and renal injury,27 was increased significantly with induction of HF in ACF rats, exacerbated in Decom group. While it was decreased markedly with ularitide treatment to ACF rats, it was restored to normal levels with concomitant OMA and ularitide treatment, implying renoprotective effect of ularitide and OMA in our model.

4.4. Effects of ularitide and OMA on renal hemodynamics and excretory functions (Table 2, Figs. 4A, B and 5A, B)

While the Com-ACF animals returned to normal UNa⁺ excretion on 7th postoperative day, the Decom-ACF exhibited avid Na retention throughout the experimental period, and deteriorated renal functions as indicated from increased plasma cystin C level and decreased GFR compared to sham control group.

Similar differences between Com and Decom-ACF rats were also observed in the urinary flow rate and FENa.

In Decom-ACF rats, plasma and urinary cGMP levels increased slightly (1.7 and 1.1-fold respectively) compared with the marked increase in the Com-ACF rats (2.9 and 1.3 respectively) versus the sham control group. This level of cGMP in Decom group not matched with the 6-fold increase in ANP versus 3.4-fold in Com-ACF, reflecting attenuated ANP in the Decom-CHF rats, but it was closely paralleled by the RAAS over activity.

Our results pointed out to the significant differences in renal responses to ularitide in Com versus Decom-ACF rats, as suggested from the differential natriuretic and diuretic response between the two subgroups of ACF rats compared to the sham control.

Rats with Com-ACF in which ularitide was administered displayed a significantly natriuretic and diuretic response.
compared with Com-ACF rats that received vehicle only. This response maximized with OMA treatment to levels statistically elevated compared to sham group.

The diuretic and natriuretic effect of both ularitide and OMA was accompanied by parallel effects on GFR, FENa excretion, CGMP with preserved renal functions.

Ularitide treatment to Decom-ACF rats induced significant but limited natriuretic effect failed to restore normal level of Na excretion.

The blunted response to urodilatin in the Na-retaining animals was associated with a marked increase in PRA and plasma aldosterone levels compared to sham control rats.

To gain a more detailed insight in the possible role of the attenuated response of ularitide in decompensated stage of CHF, in spite of the relative resistance to degradation by NEP, the OMA was administered.

The dual ACE and NEP inhibition by OMA, restored the renal response to ularitide treatment and significantly improved the natriuretic/diuretic action of ularitide in Decom-ACF rats, as represented by dramatic increase in UNa, FENa excretion, GFR, urinary flow rate and significant improvement in renal function. The improvement in the ularitide' natriuretic and diuretic response after OMA treatment was associated with a suppression of the previously elevated plasma aldosterone.

The present study suggests that increased activity of the RAAS may serve as important determinant factor in renal Na handling. This notion is further supported by the demonstration that OMA administration led to an impressive natriuresis in the Decom-ACF rats with Na retention converting them into animals that compensated and returned to normal sodium balance. Thus renal responsiveness to NPs in this experimental model of HF appears to be greatly influenced by the activity of the RAAS.

4.5. Effect of ularitide/OMA on body, organs weights (Table 1) in ACF rats

Both lung weight (LW) and heart weight (HW) versus body weight (BW) were used as indices of pulmonary congestion and cardiac hypertrophy, generally considered as two indices of the HF severity. While liver weight to BW was used as an index of systemic congestion.

With the surgical induction of HF, the Decom-ACF rats exhibited significant decrease in their BW compared to sham. Total and relative heart weights were elevated in rats with Comp-ACF (49.7%, 48.9%) and increased further in Decom-ACF (106.9%, 109.4%) versus sham rats, respectively.

In the Decom-ACF rats, the total and relative lung weights were increased by 83.6% and 85.1%. Similarly the total and
relative liver weights were increased by 17.6% and 19.1% respectively.

Although the total and relative lung and liver weights, in Com-ACF rats were comparable to those of the sham group, the Decom-ACF group had marked cardiac hypertrophy, pulmonary and liver congestion confirming decompensated HF.

Ularitide treatment to the Comp-ACF rats blocked the development of cardiac hypertrophy to level comparable to that of both OMA/ularitide and sham rats, while ularitide treatment of Decom-ACF attenuated these HF induced pathological changes with 27.9% reduction in the relative HW, maximized by concomitant OMA/ularitide treatment to 48.6% reduction.

It is evident from our results that ularitide treatment results in significant attenuation of both pulmonary and systemic congestion as denoted from decreased lung and liver weighs/body weight ratios. An effect was amplified by OMA treatment.

4.6. Effect of ularitide and OMA on cumulative survival curves (Fig. 6), in ACF rats

The ACF procedure was associated with 13% early (≤7 days) mortality, occurring mostly within the first 48 h, and these animals were excluded from all analyses.

One hundred and five animals survived the perioperative period and entered the study.

In sham control and OMA/ularitide treated Com-ACF groups, survival was 100% (15/15) till termination of study. In Com-ACF and ularitide treated Com-ACF groups, the survival was 73% (11/15) and 93% (14/15) respectively, while in Decom-ACF, the survival was dramatically reduced to 0.47% (7/15) to be improved in ularitide treated Decom-ACF to 73% (11/15) and markedly elevated with both OMA/ularitide treatment of Decom-ACF rats to about 93% (14/15).

The dual inhibition of both (ACE) and NEP together with ularitide improved survival rate even more in ACF rats.

5. Discussion

The primary objectives in the treatment of patients with HF are to improve quality of life and increase survival. Administration of NPs and maneuvers that enhance or restore their cellular actions or prevent their degradation continues to be evaluated for therapeutic efficacy in HF.

A major goal of the present study has been to evaluate the efficacy of ularitide and OMA, two of the most promising agents currently being studied, in ACF induced CHF, under different condition of compensation.

Similar to previous studies, ACF induction in the present study produced the broad neurohormonal activation, sodium-retaining hallmarks of CHF, with reduced renal
function, elevated relative organs weights and biomarkers of organ injury, that were more evident in Deco-ACF rats.

Similar to our results with ACF induction, the rats are classified to Com and Decom, and most HF patients maintain normal Na balance when maintained on low salt diet, but about 50% developed positive Na balance when fed a normal salt diet. These differences between Com- and Decom-CHF patients or animals are probably due to major differences in their hormonal status.31

A common feature of both the CHF patients and experimental animals with sodium retention10,29,31and the Decom-rats with ACF in the present study was the activation of the RAAS. It is now abundantly clear that despite early activation of the RAAS in CHF is considered as a counter-regulatory

Figure 5b  Effect of ularitide/OMA treatment on FNa (A, B), in Com and Decom ACF rats respectively. Values are mean ± SD.  
*p < 0.05 vs. Sham.  
*p < 0.05 vs. Com-ACF.  
*p < 0.05 vs. Ularitide treated Com-ACF.  
*p < 0.05 vs. De-ACF.  
*p < 0.05 vs. Ularitide treated Decom-ACF.  
GFR; glomerular filtration rate, FNa; fractional Na excretion, Com; compensated, Decom; decompensated, ACF; Aorto-caval fistula, OMA; omapatrilat.

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<th>Table 1</th>
<th>Effect of ularitide and OMA on body and organ weights in ACF-induced CHF rats.</th>
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<td></td>
<td>Sham</td>
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<tr>
<td>BW(g)</td>
<td>269.2 ± 4.237</td>
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<tr>
<td>HW(mg)</td>
<td>713.90 ± 4.771</td>
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<tr>
<td>LW(BW)(mg)</td>
<td>2.652 ± 0.086</td>
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<tr>
<td>Liver Wt.(mg)</td>
<td>973.4 ± 9.559</td>
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<tr>
<td>Liver Wt./BW (mg/g)</td>
<td>3.619 ± 0.165</td>
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<td>8206 ± 30.42</td>
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<td>30.48 ± 1.50</td>
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<td>AC, aorto-caval fistula; BW, Body weight; HW, Heart weight; Wt, wet weight. Com, compensated, Decom, decompensated, ACF; Aorto-caval fistula, OMA; Omapatrilat. CHF, congestive heart failure. Values are mean ± SD.</td>
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* p < 0.05 vs. Sham.  
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p < 0.05 vs. Ularitide treated Decom-ACF.
response directed at maintaining renal and major body organ perfusion, numerous studies implicate persistent RAAS activation in the derangement of renal and cardiac function, by enhancement of systemic vasoconstriction, promotion of other neurohormonal systems such as AVP and salt and water retention by the kidney, in addition to its direct deleterious actions on the myocardium.\textsuperscript{32}

The significant elevation of ANP observed in our study with the Com-ACF group that accentuated with the Decom-ACF, considered to be a hallmark of neurohumoral activation in CHF. This is consistent with previous data concluded that NPs concentrations are correlated with the degree of hemodynamic compromise and their high concentrations predict poor long-term survival.\textsuperscript{33} The elevated ANP plasma levels in ACF induced HF groups mostly attributed to enhanced synthesis and release of the hormone, not only by atria but also by recruitment of ventricular myocytes induced by volume loading\textsuperscript{4} rather than decreased clearance.\textsuperscript{34}

The elevated ANP is viewed as an important adaptive mechanism that helps unload the failing myocardium.\textsuperscript{4} The importance of the NPS in the CHF is further underscored by key studies demonstrated that elimination of the NP action via the use of the ANPR blocker or genetic deletion\textsuperscript{35} or surgical removal of atrium, disrupts cardiac performance and renal responses to volume loading\textsuperscript{4} rather than decreased clearance.\textsuperscript{34}

In spite of the remarkable activation of NP system in Com and Decom-ACF rats and the ability of NPs to counter the effects of the vasoconstrictor/antinatriuretic system, the two groups differed markedly in their renal responses with the Decom-ACF group displayed the salt and water retention, suggesting emergence of renal resistance to ANP, as documented from the attenuated plasma level and renal cGMP production and this could represent a critical turning point in the development of salt retention, edema formation and progression from a compensated to a decompensated state in CHF.\textsuperscript{2}

The exact mechanisms that mediate the renal resistance to NPs in overt CHF most likely are multifactorial. It is probable that activation of the renin-angiotensin axis may overcome the natriuretic effects of ANP. The markedly elevated Ang II observed in Decom-ACF rats, appears to impede the action of ANP by altered ANP intracellular transduction signal via reduced production of cGMP, or increased cGMP degradation by Ang II induced upregulated renal cGMP Phosphodiesterase (PDE) activity,\textsuperscript{37} or inducing downregulation of the ANPRs, aggravated by the chronically elevated plasma ANP levels.\textsuperscript{4,10}

It has been demonstrated that the expression and activity of the NEP are enhanced in experimental models of HF with accelerated NP degradation and decreased renal ANP availability.\textsuperscript{38} This decreased responsiveness leads to enhanced local actions of Ang II and the sympathetic nervous system in the kidney, resulting in salt retention and further deterioration of cardiac function.\textsuperscript{4}

The renal resistance to ANP could be explained by the possible appearance of abnormal circulation and inadequate secretory reserve of NPs associated with the decompensated HF.\textsuperscript{39} This was supported by Bialik et al.,\textsuperscript{30} who demonstrated significant alterations in the normal maturation of the NP granules and accelerated premature release of the hormonal content into the circulation only in the decompensated ACF rats, while they were comparable in compensated ACF and sham control groups.

The impaired renal functions with induction of HF, as reflected from reduced GFR observed in ACF rats, are consistent with the results obtained previously in experimental HF models.\textsuperscript{40} The mechanisms responsible for the high prevalence of renal dysfunction in HF populations are complex. It is attributed mostly to marked increased renal vascular resistance
and reduction in renal blood flow (RBF) as a consequence of the forward failure with reduction in the cardiac output, while several reports suggested increased venous pressure to be the culprit hemodynamic lesion to account for worsening renal function in chronic advanced HF patients.

The Ang II mediated increase in the efferent arteriolar resistance is critical for preservation of the GFR in the presence of reduced RBF. Because of the intense efferent arteriolar vasoconstriction, further compensation isn’t possible if RPP falls as a result of systemic hypotension, causing sharp decline in GFR. Secondary to the altered glomerular function and as a direct response to neurohumoral changes, the fractional reabsorption of filtered Na increased at the level of proximal tubule. Similarly in distal tubule and collecting ducts, the elevated Ang II and aldosterone levels, respectively, enhance activities of the NaCl co-transporter and the distal Na⁺ channel (ENaC).

In the Com-ACF rats as well as in the initial compensated phase of HF patients, the maintenance of the Na balance has been attributed in part to elevated NP levels. While, the Decom-ACF rats displayed Na and water retention despite the expansion of the ECF and the full activated NPs. The blunted natriuresis appears to be attributed to hemodynamic, neural alterations in HF and largely determined by the balance between two antagonistic hormonal systems: RAAS and ANP, rather than the action of a single system, where the signals from several opposing as well as synergistic systems integrated in the kidney to promote abnormal sodium and water reabsorption.

The plasma concentrations and urinary excretion of the second messenger, cGMP were significantly elevated in Com-ACF group as its levels correlate well with ANP-induced natriuresis and diuresis. While in the Decom-ACF rats, its levels failed to parallel the marked increase in ANP level. This is consistent with previous studies in overt experimental and human CHF, reported that the NP/cGMP signaling pathways are impaired with attenuated glomerular and urinary cGMP excretory capacity due to renal resistance to ANP and that such impairment may contribute to the progression of cardiorenal dysfunction in CHF.

Our results revealed marked improvement in the renal functions in ACF rats treated with ularitide accompanied by marked natriuresis and diuresis. The favorable renal effects of ularitide are probably due to both renal hemodynamic and direct tubular actions.

Besides increasing RBF through vasodilation of the renal vasculature, ularitide stimulates dilatation of afferent and constriction of efferent renal arterioles, leading to increased glomerular hydrostatic pressure and glomerular filtration. Furthermore the ularitide inhibits the Ang II-induced mesangial cell contraction via the accumulation of cGMP leading to mesangial cells relaxation thereby increases the effective surface area for filtration thus increasing the ultrafiltration coefficient. GFR improved more by inhibiting tubuloglomerular feedback.

Moreover, the ularitide has direct tubular actions, through acting in a paracrine mechanism. It can inhibit Ang II-stimulated sodium and water transport in proximal convoluted tubules. In cortical collecting ducts, it inhibits tubular water transport by antagonizing the action of vasopressin.

The increase in cGMP in ACF rats especially Com group reflects the action of ularitide on guanylate cyclase-coupled

| Table 2 Effect of ularitide and OMA on plasma cyst- C, plasma and urinary CGMP in ACF- induced CHF rats. |
|---------------------------------|---------------------------------|---------------------------------|
| Com-ACF | Ularitide treated | Ularitide/OMA treated | Decom-ACF | Ularitide treated | Ularitide/OMA treated |
| Plasma Cyst- C (µg/ml) | 1.5 ± 0.16 | 1.89 ± 0.144 * | 1.61 ± 0.107*+ | 2.934 ± 0.096* | 2.28 ± 0.17*@
| Plasma CGMP (Pmol/L) | 3.952 ± 0.23 | 10.608 ± 0.79 * | 16.3 ± 0.973*+ | 20.11 ± 1.101*+ | 7.006 ± 0.87* |
| Urinary CGMP excretion (Pmol/min) | 146.5 ± 156.21 | 2239.3 ± 141.50 | 2719.43 ± 145.06 | 4292.53 ± 136.33 | 2022. ± 149.09 |

ACP; aortocaval fistula; CHF; congestive heart failure; OMA; omapatrilat; CGMP, cyclic guanosine monophosphate; Cyst- C, cystin C; Com; compensated; decom; decompensated. The mean (horizontal line) and individual values are shown in each experimental group. * p < 0.05 vs. Sham. \* p < 0.05 vs. Com-ACF. # p < 0.05 vs. Ularitide treated Com-ACF. \# p < 0.05 vs. De-ACF. *\* p < 0.05 vs. Ularitide treated Decom-ACF.
receptors in the vessels and the kidneys. Perhaps ularitide stimulates both luminal and basolateral receptors, increasing the intracellular cGMP level, which reduces apical Na⁺-channel activity either by binding directly to the amiloride-sensitive channel or by stimulating cGMP-dependent protein kinase.

With the increased GFR observed with ularitide, one may speculate that this resulted in the greatest amount of ularitide being filtered from the plasma and presented to renal tubules, resulting in the greatest increase in urinary cGMP excretion and natriuresis.

In accordance with our results, ularitide treatment of ACF-induced HF rats and dogs, models, demonstrated significant dose-dependent increases in urinary flow, GFR, urinary sodium and cGMP excretion with improved hemodynamic parameters.

Of special interest is our finding that ularitide given to the Decom HF rats induced mild but significant natriuretic and diuretic responses with improved GFR, while the endogenous ANP failed in spite of its marked elevation. This may be explained by the fact that ularitide exhibited higher resistance to NEP, which is located mainly in the brush border of the proximal tubule. Thus, in contrast to ANP, ularitide is more likely to reach the inner medullary collecting duct to bind to the NPR-A and inhibits sodium reabsorption.

Moreover these results imply that ANF refractoriness in Decom group may not be simply due to downregulation or reduced affinity of NPR-A receptor, given that ularitide as an agonist to this receptor possesses more potent actions.

Indeed studies evaluating the effects of ularitide in ADHF patients (SIRIUS I and II) have shown favorable effects on hemodynamic, neurohumoral and symptomatic profiles accompanied by a steep increase in cGMP without any compromise in renal function despite a modest and dose-dependent decrease in BP. Moreover, a larger Phase III trial is currently ongoing, evaluating the efficacy and safety of ularitide in ADHF patients (TRUE-AHF).

Indeed, dual ACE and NEP enzyme inhibition with OMA treatment in Com group maximizing ularitide beneficial effects, while restoring renal effect of ularitide in the Decom-ACF group, resulted in a dramatic natriuresis, associated with diuretic responses with improved GFR, while the endogenous ANP failed in spite of its marked elevation. This may be explained by the fact that ularitide exhibited higher resistance to NEP, which is located mainly in the brush border of the proximal tubule. Thus, in contrast to ANP, ularitide is more likely to reach the inner medullary collecting duct to bind to the NPR-A and inhibits sodium reabsorption.

The favorable effects obtained with concomitant OMA/ularitide treatment of ACF groups, could be attributed to ACE I by OMA, reversing the previously mentioned deleterious Ang II induced renal effects, leading to drastic natriuresis, diuresis, and improved renal functions observed in ACF rats.

The favorable renal actions of OMA could be explained in part by enhancing the NPs via its NEP-inhibiting properties, as indicated by the markedly increased urinary cGMP excretion and confirmed by previous in vivo studies reported that the cardiorenal actions of OMA mediated in part by endogenous NPs, and were markedly attenuated by a NPR-antagonist.

Previous studies of experimental model and clinical trials of CHF, have reported that OMA was superior to selective NEP inhibitor, or the ACE inhibitor, in promoting Na excretion, GFR, reduced the afterload, myocardial wall stress and improved cardiac function. In the IMPRESS (Inhibition of Metalloproteinase in a Randomized Exercise and Symptoms Study in Heart Failure) trial, OMA was well tolerated, and led to a better clinical status and lower incidence of the combined mortality/morbidity end point compared with lisinopril, with preserved renal functions.

The lack of increase in plasma ANP after OMA administration was probably due to hemodynamic improvement.

Given that the NEP degrades other vasodilator peptides, we cannot exclude the role of these peptides in the potentiated effects of OMA in the CHF group.

Plasma and urinary cGMP, a marker of the renal activity of the NPs increased significantly in the Com-ACF group with ularitide and further accentuated with OMA.

The increased plasma and urinary cGMP levels observed in OMA/ularitide treated Decom-ACF rats, confirmed OMA’s efficiency in NEP inhibition, reflected enhanced sensitivity and potentiated effects of NPs, with accumulation of cGMP, possibly through blocking the Ang II mediated increases in intracellular cGMP.

When the heart fails to balance VR with CO, the decompensation occurs, that is confirmed through marked cardiac hypertrophy and injury together with systemic and pulmonary congestion, contributing to increased morbidity and mortality. However the congestion may be driven predominantly by impaired renal reserve rather than by progressive cardiac insufficiency.

The cardiac hypertrophy that accompanies HF, is initially compensatory for an increased work load; however, prolonged hypertrophy can become detrimental, and constitute a major component of cardiac decompensation and severe injury and a major risk factor for morbidity and mortality. It occurs in response to a wide variety of hemodynamic and humeral stimuli triggered by elevated Ang II which exerted a mechanical stress on myocardium due to the systemic Ang II – mediated increase in the afterload and mediated by local Ang II, further aggravated by the aldosterone that acts directly on the myocardium, inducing the structural remodeling of the interstitial collagen matrix.

Concerning the obtained antihypertrophic effect of ularitide, that was enhanced by OMA in ACF rats, it may be secondary to improvement of the hemodynamic status by reducing the pre- and afterload due their potent diuretic effect, and mediated by RAAS suppression and enhanced NPs/GC-a/cGMP/protein kinase type I signaling that negatively regulates cardiac hypertrophy via inhibition of CYP11B2 (aldosterone synthase) mRNA expression and the calcineurin-nuclear factor of activated T cells signaling pathway.

The role of ongoing myocardial injury in HF has been established with a strong association between elevated troponin levels and poor clinical outcomes. Similar to previous results, the CHF induction, induced mild elevation of cTnT, in Com-ACF that exacerbated in Decom-ACF group, which may be related to hemodynamic and/or neurohumoral abnormalities.

Ularitide and OMA treatment was associated with reduction of biomarkers indicative of myocardial and renal injury.

Consistent with previous results decreased plasma NT-proBNP concentrations observed in ularitide and OMA treated ACF groups, secondary to improve hemodynamic and reflect reduced cardiac filling pressures that lower ventricular wall stress.
In the Decom-ACF rats, backward failure was evident with increased liver and lung weights to BW, indicating systemic and pulmonary congestion, associated with disease severity. Of special interest in our study, ularitide/OMA treatment was able to manage congestion effectively, as shown not only by the significant reduction in relative organ weights but also by the reduction of NPs levels. It is probable that the prevention of organ damage, relief from congestion and preserved renal functions may be associated with lower mortality of ACF rats, when treated with ularitide and OMA.

6. Conclusion

Treatment of ACF rats with both ularitide and OMA considerably improved the animals’ survival rate and inhibited the development of renal dysfunction and organ injury. These protective actions were associated with significant suppression of the RAAS with simultaneous unmasking and potentiation of the NPs effects. So they may become therapeutic options to improve the health status and clinical outcomes of patients with CHF especially acute decompensated stage.

Conflict of interest

The authors declare that they have no conflict of interest.

References


