Effects of Chronic Rosiglitazone Treatment on Renal Handling of Salt and Water in Rats With Volume-Overload Congestive Heart Failure

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Background—The side effects of fluid retention and edema of the thiazolidinedione (TZD) class of peroxisome proliferator-activated receptor-γ agonists limit their use in patients with congestive heart failure (CHF). The present study aims to explore whether chronic treatment with the TZD compound rosiglitazone (RGZ) is associated with worsening of salt and water retention in male Sprague-Dawley rats with aorto-caval fistula, an experimental model of volume-overload CHF.

Methods and Results—The effects of oral RGZ (30 mg/kg per day for 4 weeks) in CHF rats on plasma volume, cumulative sodium excretion, renal expression of Na\(^+\) channels and transporters, and selected biomarkers of CHF were compared with those in CHF rats and sham-operated control rats treated with vehicle only (n = 7 to 10). Additionally, the response to acute saline loading (3.5% of body weight) was evaluated after 2 weeks of treatment by renal clearance methodology. Chronic RGZ treatment caused no further increase in plasma volume compared with vehicle-treated CHF rats. Moreover, no increase in renal expression of Na\(^+\) transport-linked channels/transporters was observed in response to RGZ. Cumulative sodium excretion was enhanced in CHF rats after RGZ and by another TZD compound, pioglitazone. In response to saline loading, RGZ-treated animals displayed a higher natriuretic/diuretic response than did vehicle-treated rats. Chronic RGZ treatment was not associated with any deterioration in selected biomarkers of CHF, whereas indices of cardiac hypertrophy and blood pressure were improved.

Conclusions—Chronic RGZ treatment was not associated with worsening of fluid retention or cardiac status in rats with experimental volume-overload CHF. Rather, RGZ appeared to improve renal handling of salt and water in rats with CHF. (Circ Heart Fail. 2011;4:345-354.)

Key Words: thiazolidinedione ■ aorto-caval fistula ■ renal sodium excretion ■ ECF volume expansion ■ rat

Rosiglitazone (RGZ) is a member of the thiazolidinedione (TZD) class of compounds that act as agonists of peroxisome proliferator-activated receptor-γ (PPAR-γ). Activation of PPAR-γ exerts important metabolic effects by regulating the expression of genes involved in glucose and lipid metabolism. Oral RGZ is highly effective in improving insulin sensitivity and the metabolic abnormalities of type 2 diabetes mellitus (T2DM). However, the clinical benefits of TZDs are hampered by the concomitant adverse effects of fluid retention and peripheral edema. These were reported in 4% to 7% of TZD-treated patients and in up to 15% in those who receive concurrent insulin therapy. Moreover, the overall cardiovascular safety of these drugs has been questioned on the basis of observations of increased incidence of heart failure and cardiac ischemic events in RGZ-treated patients. In particular, the increase in cardiac events led recently to more restrictive measures on RGZ use in patients with T2DM. Indeed, the original guidelines of the American Heart Association (AHA) prohibit the use of TZDs in patients with established congestive heart failure (CHF) (New York Heart Association functional class III-IV) and further advise caution when treating T2DM patients at risk for heart failure. Consequently, the effects of TZD treatment in patients with preexisting heart failure have not been thoroughly studied, and only limited data exist in the literature.

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Current evidence suggests that the mechanism for TZD-induced fluid retention involves primarily direct salt-retaining effects of PPAR-γ agonists on the nephron. Specifically,
although some of the studies indicated altered transporter expression or function in the distal and proximal tubules,16–18 most findings point to the aldosterone-regulated epithelial sodium channel (ENaC) in the collecting duct as the main target of PPAR-γ-mediated sodium retention.19–22 However, some contradicting evidence to these findings exists as well.23–25 The studies published so far on the effect of TZDs on renal sodium and water handling were conducted in healthy or diabetic animal models or in cell cultures, and, to the best of our knowledge, there are no reported data in experimental models of CHF. Furthermore, most of these studies examined the short-term effect of TZD treatment.

Rats with aorto-caval fistula (ACF), originally introduced by Stumpe et al.,26 serve as an experimental model of volume-overload CHF.27–29 This model closely mimics the manifestations in patients with advanced CHF, including neurohumoral activation, cardiac hypertrophy, and impaired renal handling of salt and water. Previously we reported that rats with ACF can be divided into 2 subgroups based on their ability to excrete sodium. After an initial period of salt and water retention, most animals undergo renal compensation and increase their urinary sodium excretion despite maintaining the neurohumoral “milieu” of CHF (henceforth termed “compensated”). The others continue to retain salt and water avidly and die within 7 to 12 days of pulmonary edema and “decompensated CHF.”27,29 We hypothesized that exposing rats with “compensated CHF” to chronic TZD treatment could lead to excessive sodium retention and deterioration to a decompensated state. Therefore, in the present study, we evaluated the effects of chronic treatment with RGZ or vehicle on renal handling of salt and water in rats with compensated CHF. Specifically, we studied the effects of the drug on plasma volume, daily and cumulative Na\(^+\) excretion, gene expression of various renal transporters and ion channels implicated in the pathogenesis of TZD-induced salt retention, as well as on selected biomarkers of cardiac dysfunction. Additionally, the response to a dynamic natriuretic maneuver, namely, acute intravenous saline loading, was tested in CHF animals subjected to chronic treatment with RGZ.

**Methods**

A detailed methods section is provided in the supplemental material. Male Sprague-Dawley rats (Harlan Laboratories, Jerusalem, Israel) weighing 280 to 320 g (10 to 11 weeks old) were housed in individual metabolic cages and fed a standard rodent diet (containing 0.4% to 0.5% NaCl) and tap water ad libitum. Experiments were performed according to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) as approved by the local committee for supervision of animal experiments.

**Experimental Model**

A fistula was surgically created between the abdominal aorta and the inferior vena cava (side to side, 1.2 mm O.D.), as previously reported from our laboratory.27–29 Sham-operated rats, with laparotomy only, served as a control group. Only rats with compensated heart failure (\(U_{\text{Na}}/V\geq1200\) μEq/d) were included in the present study.

**Effects of Chronic RGZ Treatment on Renal Salt and Water Excretion and Indices of Heart Failure**

Oral RGZ (30 mg/kg per day) or vehicle were started in rats with compensated CHF 7 days after the operation, and vehicle was started in sham-operated control rats for 28 days (n=10 in each group). This relatively high dose of RGZ is in the range that was shown to produce a significant increase in extracellular fluid (ECF) volume in Zucker rats.30 Urine output and sodium excretion were monitored daily and cumulative \(U_{\text{Na}}/V\) was calculated over the experimental period. Hematocrit and body weight were measured at 8- to 10-day intervals and on the last day of experiments. At the end of treatment, mean arterial pressure (MAP) was measured and blood was collected for analyses of aldosterone, brain natriuretic peptide (BNP), adiponectin, and insulin. Both kidneys and the heart were removed and weighed and the cortex and outer renal medulla were separated and kept at –70°C for gene expression studies. To rule out agent-specific differences in the cardiovascular effects of TZD compounds,31 a similar protocol for cumulative Na\(^+\) excretion was repeated with pioglitazone (10.0 mg/kg per day for 28 days) in CHF rats and matching groups of vehicle-treated CHF and control rats (n=5 in each group).

**Plasma Volume Measurements**

Plasma volume was measured using the dye dilution technique with Evans blue in additional groups of CHF rats and control rats (n=7 to 10 each) that were treated with RGZ for 4 weeks as discussed above.

**Renal Response to Extracellular Fluid Volume Expansion**

Renal and hemodynamic response to ECF volume expansion were evaluated by clearance methodology in ACF rats treated with RGZ or vehicle for 2 weeks and sham-operated control rats (n=8 to 10). Solutions of 2% inulin in saline and of normal saline were continuously infused by separate syringe pumps. After 2 baseline urine collections, the rate of normal saline infusion was adjusted to deliver a volume equivalent to 3.5% of body weight over a 10-minute period and then returned to the original rate, followed by 4 additional clearance periods. Blood samples were taken for inulin and electrolyte analysis. Glomerular filtration rate (GFR) was equated with the clearance of inulin.

**Targeted Tissue Low-Density Gene Array**

After RNA and cDNA preparation, quantitative real-time polymerase chain reaction was performed utilizing custom-designed TaqMan low-density arrays from Applied Biosystems. Detailed experimental conditions on the TaqMan low-density array analysis were described previously.32 The comparative C\(_T\) method of relative quantification was used, using averaged values for hypoxanthine-guanine phosphoribosyltransferase, peptidylprolyl isomerase A, and glyceraldehyde 3-phosphate dehydrogenase as normalizers and the control + vehicle group mean (n=8 to 10) as calibrator.

**Analytical Methods**

Urine and plasma electrolytes were measured by flame photometry. Urine volume was determined gravimetrically. Urine and plasma inulin and plasma albumin concentrations were determined colorimetrically. Blood glucose was measured in tail vein samples by Ascensia Elite blood glucometer, and hematocrit was determined by heparinized capillary tubes. Serum aldosterone and plasma insulin were determined by commercially available radioimmunoassay kits and plasma BNP-32 and adiponectin by enzyme-linked immunosorbent assay.

**Statistical Analysis**

One-way ANOVA and 2-way ANOVA for repeated measurements were used for group comparison, as appropriate. Tukey and Bonferroni corrections for multiple comparisons were used as ANOVA post hoc tests, respectively. Repeated-measures 1-way ANOVA, followed by Dunnett multiple comparison test, was used to test significance of change from baseline values of clearance parameters within treatment groups in the volume expansion experiments. \(P<0.05\) was chosen as the significance level for all analyses. Data are expressed as mean±SE.
Table. Body Weight, Plasma Volume Markers, and Metabolic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Control + Veh</th>
<th>CHF + Veh</th>
<th>CHF + RGZ</th>
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<tbody>
<tr>
<td>Initial weight, g</td>
<td>322.8±3.8</td>
<td>318.1±3.7</td>
<td>322.4±4.4</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>371.3±7.4</td>
<td>380.8±8.2</td>
<td>395.7±8.6</td>
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<tr>
<td>% Change in weight</td>
<td>15.1±2.2</td>
<td>19.7±2</td>
<td>22.7±2°</td>
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<tr>
<td>Hematocrit, %</td>
<td>51±1.2</td>
<td>42.3±1.6°</td>
<td>43.5±0.8°</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.3±0.1</td>
<td>4.1±0.1</td>
<td>4.2±0.1</td>
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<tr>
<td>Glucose, mg/dL</td>
<td>120.7±4.4</td>
<td>99.4±6°</td>
<td>108.2±4.5</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>2.1±0.1</td>
<td>1.0±0.2°</td>
<td>1.5±0.1°</td>
</tr>
<tr>
<td>Adiponectin, μg/mL</td>
<td>3.6±0.3</td>
<td>3.6±0.5</td>
<td>13.1±1.7°</td>
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Veh indicates vehicle. Initial and final weights depict body weight at the beginning and end of 4-week treatment, respectively. Metabolic analytes were measured in plasma except for glucose, which was measured in whole-blood samples.

Data represent mean±SEM. 
*P<0.05 versus control + Veh; †P<0.05 versus CHF + Veh.

Results

The Table summarizes the data of chronic RGZ/vehicle treatment on body weight, metabolic parameters, hematocrit, and plasma albumin levels in CHF rats compared with vehicle-treated control animals. Body weight increased during the 4 weeks of treatment by 15% to 23% in the 3 studied groups. The highest gain in weight was observed in CHF rats treated with RGZ (22.7±2.0%) compared with control rats (15.1±2.0%) (15.1±2.0%) compared with control rats (22.7±2.0%) compared with control rats (15.1±2.0%). Of notice, the significant increases in body weight were due to the presence or absence of CHF and not due to treatment with RGZ in CHF rats. Similarly, blood hematocrit was lower in CHF rats treated with RGZ or vehicle compared with the control rats (P<0.05) but not between the groups of CHF rats. No consistent changes were observed in plasma albumin, nonfasting blood glucose, or insulin levels after RGZ treatment in CHF rats (Table). Plasma adiponectin, a marker of TZDs biological action, increased almost 4-fold in CHF rats treated with RGZ or vehicle compared with CHF or control rats treated with the vehicle (P<0.05, Table).

Figure 1 illustrates the effects of RGZ treatment on plasma volume estimated by the Evans blue dye distribution. There was no significant difference between RGZ- and vehicle-treated rats with CHF, and both exhibited higher (P<0.05) plasma volume compared with vehicle-treated control rats in absolute terms (Figure 1A) and when normalized to body weight (Figure 1B). Figure 2 depicts the cumulative sodium excretion during baseline, postoperative and treatment phases (Figure 2A), and repeated body weight measurements taken in parallel during RGZ and vehicle treatment (Figure 2B). A significant decrease in cumulative sodium excretion was observed in CHF rats compared with control animals, reflecting salt and water retention in rats with CHF. Thus, from the day of surgery until the last day of treatment with vehicle, cumulative sodium excretion in rats with CHF was significantly lower than in control rats (43 840±2489 μmol versus 51 010±1418 μmol, P<0.05). This decrease is commensurate with the observed increase in body weight that was higher in CHF than in sham-operated control rats (Table and Figure 2B). Interestingly, despite the more marked gain in body weight observed in CHF rats treated with RGZ (Figure 2B), these rats did not exhibit a further decrease in cumulative urinary sodium excretion compared with vehicle-treated rats (Figure 2A). In fact, cumulative sodium excretion in CHF rats treated with RGZ (50 550±1237 μmol) was higher than that observed in CHF rats treated with vehicle (P<0.05), suggesting that RGZ actually improved renal excretion of sodium and water in rats with CHF. A similar pattern was observed also with another TZD compound, pioglitazone, which had essentially the same effect on cumulative Na⁺ excretion in rats with CHF (Figure 2C) and on body weight gain (data not shown), suggesting that the observed effect on natriuresis does not depend on a specific TZD agent.

Figure 3 summarizes the gene expression data in the renal cortex and the outer medulla in the 3 studied groups. Of note, no significant increase was observed in the mRNA expression of any of the renal sodium transporters and channels examined in the present study in response to 4 weeks of treatment with RGZ in CHF rats, except for a trend of increase in cortical Sgk1 expression (see Figure 3 legend). Actually, the expression of ENaC in the renal cortex and outer medulla and of sodium chloride cotransporter (NCC) in the renal cortex tended to be slightly reduced in both groups of CHF rats compared with control rats.

The effects of chronic treatment with RGZ or vehicle in CHF rats on selected biomarkers of heart failure are shown in Figure 4. In vehicle-treated rats with CHF, circulating level of
BNP and aldosterone tended to be higher than in control rats. Cardiac/body weight ratio, an index for cardiac hypertrophy, was significantly higher (CHF+vehicle, 0.548±0.024%; control+vehicle, 0.312±0.007%; P<0.05) and MAP was lower than in sham-operated control rats (CHF+vehicle, 116.6±4.4 mm Hg; control+vehicle, 152.3±6.7 mm Hg; P<0.05). After RGZ treatment in rats with CHF, there was a slight but significant decrease in cardiac/body weight ratio (0.467±0.024%), and MAP was higher compared with non-treated CHF rats (140.9±5.1 mm Hg, P<0.05, Figure 4A). In addition, RGZ treatment in CHF was associated with a tendency to a decrease in circulating BNP levels and a significant reduction in serum aldosterone (P<0.05). The lack of detrimental effects of RGZ on cardiovascular biomarkers associated with heart failure is further supported by the cardiac gene expression data (Figure 4B). Chronic treatment with RGZ caused no further increase in myocardial mRNA expression of atrial natriuretic peptide and BNP in CHF rats, although baseline expression of these genes tended to be elevated in CHF rats compared with control rats.
Interestingly, treatment with RGZ in CHF rats caused a significant decrease in cardiac mRNA expression of angiotensin-converting enzyme (ACE), a key component of the renin-angiotensin-aldosterone system (of ~49% compared with vehicle-treated CHF rats, \( P < 0.05 \)). Finally, chronic RGZ treatment significantly increased the expression of the autoregulated PPAR-\( \gamma \) mRNA compared with vehicle-treated groups (about 2-fold, \( P < 0.05 \)). The latter change was observed also in kidney tissue samples, although to a lesser extent (data not shown).

The natriuretic/diuretic response to ECF volume expansion and related changes in GFR and MAP in the 3 studied groups are shown in Figure 5 and Figure 6. In response to saline loading (3.5% of body weight), there was a significant increase in urinary flow rate, \( U_{\text{NaV}} \), and fractional sodium excretion (%ENa), followed by a decrease in these parameters during the recovery phase (clearance periods 5 and 6, Figure 5A through 5C). However, the increase in \( U_{\text{NaV}} \) in response to saline loading in vehicle-treated CHF rats (from 0.3 ± 0.07 to 10.3 ± 1.1 \( \mu \)mol/min) was significantly lower than in vehicle-treated control rats (from 1.4 ± 0.5 to 18.2 ± 1.8 \( \mu \)mol/min, \( P < 0.05 \)). A similar pattern was observed when urinary excretory parameters were expressed as %ENa or urinary flow rate (Figure 5B and 5C). Interestingly, 2 weeks of RGZ treatment in rats with CHF significantly improved the ability of the kidney to excrete the saline load compared with vehicle-treated CHF rats (Figure 5A through 5C). This is further supported by the data comparing the cumulative sodium excretion during the entire acute experiment (baseline, saline-loading, and recovery phases) in the 3 studied groups (Figure 5D). That the RGZ-related improvement in renal handling of sodium and water cannot be attributed to parallel changes in GFR is shown in Figure 6. Saline loading was associated with a reversible decrease in GFR during the rapid infusion that was more pronounced in control rats. However, there were no significant changes in GFR in
RGZ-treated versus vehicle-treated rats with CHF (Figure 6A). Although not reaching statistical significance, it is noteworthy that MAP tended to be slightly higher in CHF rats treated with RGZ during the entire experiment compared with vehicle-treated CHF rats (Figure 6B).

**Discussion**

The present study provides novel information on the effects of chronic treatment with RGZ on renal handling of salt and water in rats with compensated CHF. Our findings demonstrate that chronic RGZ treatment was not associated with impairment in the ability of the kidney to excrete salt and water, both in CHF rats maintained on a normal salt diet and also in response to acute intravenous saline loading. If anything, the data suggest that RGZ administration was associated with “improved” handling of salt and water by the kidney in rats with experimental CHF. This is illustrated by a higher cumulative excretion of sodium with 2 different TZD compounds and a more pronounced natriuretic response to saline loading in RGZ-treated rats with CHF compared with vehicle treatment and further supported by the lack of additional increase in plasma volume in response to RGZ treatment in CHF rats. Recent data indicated that RGZ and pioglitazone may differ in their cardiovascular effects in patients, suggesting the existence of agent-specific actions of the TZD compounds. Our data demonstrate that RGZ and pioglitazone share similar effects on cumulative Na⁺ excretion in CHF rats, indicating that the improved renal handling of sodium after RGZ is not an agent-specific effect. The findings of no significant increase in plasma volume together with the lack of change in blood hematocrit indicate that when administered in the setting of an already expanded plasma volume in CHF, RGZ treatment is not associated with further accumulation of fluids in the intravascular compart-
ment. Moreover, genomic analysis indicated that chronic RGZ treatment did not cause any detectable increase in the transcription of transporters and channels linked with sodium transport in the renal cortex or outer medulla. In fact, in rats with CHF, the mRNA expression of ENaC in the renal cortex and outer medulla and of NCC in the renal cortex was slightly reduced. This finding could be attributed to the presence of heart failure, probably reflecting compensatory means to limit salt and water retention, and was not due to treatment with RGZ. Finally, RGZ treatment in rats with CHF was not associated with deterioration in cardiac function. On the contrary, several biomarkers of cardiac dysfunction and hypertrophy, such as myocardial expression of ACE, cardiac/body weight ratio, and serum aldosterone level, were significantly reduced by RGZ. Additionally, MAP was higher in CHF rats treated with RGZ. Thus, chronic exposure to RGZ in a rat model of volume-overload CHF did not result in a worsening of cardiac and renal status.

The guidelines of the American Heart Association and the American Diabetes Association restrict the use of TZDs in T2DM patients with CHF, based on the tendency to develop edema in response to these drugs, a phenomenon assumed to result in worsening of heart failure. Indeed, several studies suggested that increased salt and water reabsorption by the kidney plays an important role in generation of TZD-induced edema and implicated the collecting duct as the major site of TZD-induced sodium retention. However, data contradicting this notion are also found in the literature. In this regard, Nofziger et al reported that PPAR-γ agonists failed to enhance basal or insulin-stimulated sodium transport in a variety of collecting duct cell lines. Two other recent studies also argued against a primary role of the ENaC in TZDs-induced fluid retention and suggested an involvement of a nonselective cation channel or decreased expression of the cystic fibrosis transmembrane regulator as alternative mechanisms. Thus, the mechanism of TZD-induced fluid retention remains controversial at present. Also, in a study determining the time course of AQP-2 and ENaC response to PPAR-γ agonist administration, Tiwari et al showed increases in immunoreactive protein of AQP-2 and ENaC in the collecting duct, either directly or by an aldosterone-dependent manner. However, data contradicting this notion are also found in the literature. In this regard, Nofziger et al reported that PPAR-γ agonists failed to enhance basal or insulin-stimulated sodium transport in a variety of collecting duct cell lines. Two other recent studies also argued against a primary role of the ENaC in TZDs-induced fluid retention and suggested an involvement of a nonselective cation channel or decreased expression of the cystic fibrosis transmembrane regulator as alternative mechanisms. Thus, the mechanism of TZD-induced fluid retention remains controversial at present. Also, in a study determining the time course of AQP-2 and ENaC response to PPAR-γ agonist administration, Tiwari et al showed increases in immunoreactive protein of AQP-2 and ENaC in the collecting duct, either directly or by an aldosterone-dependent manner. However, data contradicting this notion are also found in the literature. In this regard, Nofziger et al reported that PPAR-γ agonists failed to enhance basal or insulin-stimulated sodium transport in a variety of collecting duct cell lines. Two other recent studies also argued against a primary role of the ENaC in TZDs-induced fluid retention and suggested an involvement of a nonselective cation channel or decreased expression of the cystic fibrosis transmembrane regulator as alternative mechanisms. Thus, the mechanism of TZD-induced fluid retention remains controversial at present. Also, in a study determining the time course of AQP-2 and ENaC response to PPAR-γ agonist administration, Tiwari et al showed increases in immunoreactive protein of AQP-2 and ENaC in the collecting duct, either directly or by an aldosterone-dependent manner. However, data contradicting this notion are also found in the literature. In this regard, Nofziger et al reported that PPAR-γ agonists failed to enhance basal or insulin-stimulated sodium transport in a variety of collecting duct cell lines. Two other recent studies also argued against a primary role of the ENaC in TZDs-induced fluid retention and suggested an involvement of a nonselective cation channel or decreased expression of the cystic fibrosis transmembrane regulator as alternative mechanisms.

Figure 5. Effects of 2-week RGZ treatment on urinary Na\(^{+}\) clearance parameters in response to ECF volume expansion. Data represent mean±SEM of n=8 to 10 rats in each group. A. Absolute Na\(^{+}\) excretion rate. B. Urinary flow rate. C. Fractional Na\(^{+}\) excretion rate (%ENa). D. Cumulative Na\(^{+}\) excretion (AUC of A). BL indicates baseline; VE, volume expansion; and U3–6, urine collection 3 to 6. BL values represent average of urine collections 1 to 2. \(*P<0.05\) for CHF+Veh versus control+Veh; \(#P<0.05\) for CHF+RGZ versus CHF+Veh. Veh indicates vehicle.

Figure 6. Effects of 2-week RGZ treatment on GFR (A) and MAP (B) in response to ECF volume expansion. Data represent mean±SEM of n=7 to 10 in each group. Baseline values represent average of urine collections 1 to 2; volume expansion (VE) values represent average of urine collections 3 to 4; and recovery values represent average of urine collections 5 to 6. \(*P<0.05\) versus baseline; \(#P<0.05\) versus control+Veh. Veh indicates vehicle.
aldosterone, which promotes Sgk1-mediated renal sodium retention, was lower in RGZ-treated than in vehicle-treated rats with CHF.

The findings of the present study are of interest in several respects. Despite a significant increase in body weight that was the highest in rats with CHF treated with RGZ, our study suggests that administration of RGZ was not associated with worsening of fluid retention and actually promoted sodium and water excretion by the kidney in CHF rats. This seeming paradox could be explained in part by the effect of chronic RGZ treatment on adipogenesis and appetite. Although in some studies the TZD-related increase in body weight was attributed primarily to fluid retention and edema formation, there is compelling evidence that other factors may be involved as well. It is known that PPARγ is a key regulator of adipogenesis and activates genes involved in lipid storage. Recently, Takazawa et al35 reported that RGZ increased the expression of very low-density lipoprotein receptor as well as body weight and white adipose tissue in obese mouse models but failed to increase body weight and adipose tissue in very low-density lipoprotein receptor–deficient mice. It is possible that the tendency toward a higher body weight in CHF rats treated with RGZ compared with vehicle-treated CHF rats may be in part due to a RGZ-induced adipogenesis. Thus, the finding that the most significant increase in body weight in the CHF+RGZ group occurred during the latter phase of the treatment period was not accompanied by a parallel decrease, but rather with an increase in cumulative Na+ excretion, may suggest that the increment in body weight is not primarily due to fluid retention. However, we cannot completely exclude that peripheral edema accounts for some of the weight gain, as it has not been directly assessed. Furthermore, in the present study, we did not monitor daily food intake. Therefore, we cannot rule out the possibility that improved appetite in RGZ-treated rats with CHF caused a higher sodium intake, which may have contributed to the increased daily excretion of sodium. Nevertheless, such a possibility does not contradict the notion that RGZ did not cause further sodium retention by the kidney. Certainly, it cannot explain the “normalization” of the natriuretic response to acute saline loading in RGZ-treated CHF rats, if indeed the drug had a potent salt-retaining property.

The mechanism(s) of the improvement of renal handling of salt and water induced by RGZ were not elucidated in the present study. Nevertheless, several preliminary findings such as the improvement in cardiovascular biomarkers, suggest that this may be due to enhancement of cardiac performance induced by the drug. In that respect, the finding that chronic RGZ treatment was associated with a significant reduction in myocardial expression of ACE in CHF rats is of particular interest. To our knowledge, this effect of RGZ is novel and was not described previously. Indeed, renal retention of sodium and cardiac hypertrophy in rats with CHF induced by ACF are highly dependent on the activity of the renin-angiotensin system and may be reversed or prevented by treatment with ACE inhibitors or angiotensin receptor blocker.27,28 Furthermore, Geng et al16 demonstrated that RGZ treatment in rats with myocardial infarction inhibited myocardial angiotensin II and aldosterone generation and improved cardiac remodeling. In fact, several studies with TZDs in experimental models of cardiac injury, in particular myocardial ischemic injury in normal rats as well as in diabetic rodent models, demonstrated potential cardiovascular protective actions of these drugs.37–39 These beneficial effects were attributed in large to the anti-inflammatory properties of the TZDs and the amelioration of oxidative stress. Whether such a mechanism could also contribute to the improvement in salt and water excretion by the kidney remains to be elucidated in future studies. Although our findings suggest that the enhancement in sodium and water excretion by the kidney may be due to a central hemodynamic effect, we cannot rule out the possibility that RGZ could act through a direct renal tubular mechanism to improve renal sodium handling. We did, however, find that RGZ treatment was not associated with any corresponding increase in GFR.

In that regard, Goenka et al40 have recently reported that chronic treatment with TZDs improved the natriuretic response to water immersion–induced volume expansion in T2DM patients without clinical cardiovascular disease. Thus, the mechanism for these effects requires further evaluation. Moreover, it is entirely possible that TZDs may also induce peripheral edema, and consequently weight gain, primarily by a nonrenal mechanism, such as a PPAR-γ–related increase in vascular permeability. Accordingly, Sotiropoulos et al41 reported a possible mechanism in which RGZ increased Evans blue dye permeability in adipose tissues and the retina of diabetic rats through a diacylglycerol protein kinase C–dependent pathway.

Finally, in the current study we asked specifically whether the presence of CHF “milieu” renders the animals more vulnerable to the sodium- and water-retaining effects of RGZ. We could find no evidence that chronic treatment of RGZ caused a further deterioration in cardiac function or induced intravascular fluid retention beyond that found in rats with uncomplicated CHF. Yet, the clinical significance of these findings should be interpreted with caution, especially in view of several studies in patients suggesting the opposite conclusion (see review by Erdmann et al42). The reasons for such a discrepancy are not clear and may be due to species differences or the presence of T2DM and insulin resistance in patients treated with TZDs. Our attempts to induce CHF in Zucker rats, with insulin resistance and obesity, failed because of the extremely high mortality rate that precluded us from completing 4 weeks of treatment with RGZ. Nevertheless, based on the findings in our model, the possibility that the combination of RGZ with uncomplicated CHF is less detrimental than thought before deserves additional investigation, certainly in view of the beneficial metabolic effects of these drugs.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Rosiglitazone (RGZ), a peroxisome proliferator-activated receptor-γ analog of the thiazolidinedione (TZD) class of insulin sensitizers, has been used successfully for more than a decade to control blood glucose levels in patients with diabetes mellitus. However, the use of TZDs in diabetic patients with established congestive heart failure (CHF) has been prohibited by both the American Heart Association and the American Diabetes Association because of their tendency to induce fluid retention and edema formation. The latter phenomenon, which may aggravate the severity of CHF, is thought to be due to direct renal tubular salt-retaining effects of peroxisome proliferator-activated receptor-γ agonists. The present study provides evidence that chronic treatment with RGZ in rats with aorto-caval fistula, an experimental model of volume-overload CHF, with a propensity to salt and water retention by the kidney, was not associated with a further increase in renal sodium and fluid retention. In fact, chronic RGZ administration resulted in an improvement in renal handling of salt and water and in the ability of CHF rats to respond favorably to acute intravenous saline load. Furthermore, there was no evidence for transcriptional activation of key renal sodium transporters/channels or worsening in several CHF severity parameters. Our data suggest that when given in the setting of an already-expanded extracellular volume, TZDs do not necessarily cause further deterioration in renal or cardiac status in nondiabetic CHF rats. However, these findings should be interpreted with caution in light of the recent reports of increased incidence of cardiovascular ischemic events and mortality after RGZ treatment in diabetic patients.
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Supplemental Material
Full methods

Male Sprague-Dawley rats (Harlan Laboratories, Jerusalem, Israel), weighing 280-320g (10-11 weeks-old) were housed in individual metabolic cages and fed a standard rodent diet (containing 0.4-0.5% NaCl) and tap water *ad libitum*. Experiments were performed according to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996) as approved by the local institutional committee for supervision of animal experiments.

Experimental model

A fistula was surgically created between the abdominal aorta and the inferior vena cava (side to side, 1.2 mm O.D.) under pentobarbitone anesthesia (70 mg/kg BW, i.p), as reported previously from our laboratory.\(^1,2,3\) Sham-operated rats, with laparotomy only, served as a control group. Based on their daily Na\(^+\) excretion ($U_{Na}^+$) in the following 7 days, rats with ACF were divided either to compensated ($U_{Na}^+>1200 \ \mu\text{Eq/day}$) or decompensated ($U_{Na}^+<200 \ \mu\text{Eq}$) heart failure.\(^1,2\) Only rats with compensated heart failure were included in the present study.

Effects of chronic RGZ treatment on renal salt and water excretion, and indices of heart failure

Seven days after the operation, rats with compensated CHF were started on either RGZ treatment (30 mg/kg/day, dissolved in 1 ml of vehicle solution consisting of: 0.4 ml 5% Tween-80, 0.25 ml 2% methyl-cellulose and 0.35 ml DDW) or the vehicle only (1 ml/day), and sham-operated controls were started on vehicle, by oral gavage for 28 days (N=10 in each group). This relatively high dose of RGZ is in the range
that was shown to produce a significant increase in ECF volume in Zucker rats.\textsuperscript{4} Urine output and sodium excretion were monitored daily and the cumulative $U_{Na}^{\text{t}}$ was calculated over the experimental period. Hematocrit and body weight were measured at 8-10 day intervals and on the last day of experiments. At the end of treatment, the rats were anesthetized by inactin (sodium thiobutabarbital, 100 mg/kg BW i.p, Sigma Aldrich Co., St Louis, MO). Mean arterial pressure (MAP) was measured by a transducer (model 1050.1, UFI, Morro Bay, CA) through a carotid arterial line (PE-50) and recorded (B.P instrument, model 50110, Stoelting Co., Wood Dale, IL). Blood was collected by cardiac puncture into a heparinized syringe and plasma was separated by centrifugation and stored at -70°C until analysis. Both kidneys and heart were removed, washed in cold saline, blot-dried by paper and weighed. The outer renal medulla and the cortical portions were separated. Renal and cardiac tissue samples were kept at -70°C for gene expression studies.

In view of the recent study by Graham et al\textsuperscript{5} that suggested the existence of agent-specific differences in the cardiovascular effects of TZD compounds, a similar protocol for cumulative Na$^{+}$ excretion was repeated with another TZD compound, pioglitazone (10.0 mg/kg/day, for 28 days, dissolved in the same vehicle), in CHF rats and matching groups of vehicle-treated CHF and control rats (N= 5 in each group).

**Plasma volume measurements**

Plasma volume was measured using the dye dilution technique with Evans Blue.\textsuperscript{6} Briefly, groups of CHF rats and controls (N=7-10 each) were treated for 4 weeks and then anesthetized by pentobarbitone sodium (as above). A baseline blood sample (~200 µl) was taken from the arterial line for a blank and Hematocrit measurement, and then ~0.2 ml of a 0.5 g% solution of Evans Blue (Sigma) in saline was injected
slowly into the vein. Arterial blood samples were taken repeatedly at 10, 20, 30, 45, 60 min. Plasma duplicates of 20 µl were recovered in 180 µl PBS and absorbance was measured at 620 nm. Evans Blue concentration was calculated using a standard curve (0-120 µg/ml) prepared in donor rat plasma. The log-concentrations were extrapolated to time 0 to correct for dye disappearance and the value was used for calculation of the dye’s volume of distribution.

**Renal response to extracellular fluid (ECF) volume expansion**

The effects of RGZ treatment for 2 weeks on renal and hemodynamic response to ECF volume expansion were evaluated by clearance methodology in rats with ACF or sham operation (N=8-10). Rats were anesthetized with inactin (100 mg/kg BW, i.p.) and prepared for clearance studies as described previously. Two solutions, of 2.0% inulin in saline and of normal saline, were continuously infused by two syringe pumps (New Era Pump Systems, Farmingdale, NY, USA) at a total rate equivalent to ≈1.0% of BW per hr. After two baseline urine collections of 30 min the rate of normal saline infusion was adjusted to deliver a volume equivalent to 3.5% of BW, during a 10 min period and then returned to the original rate, followed by 4 additional clearance periods. Blood samples (300 µl) were taken every second clearance period, separated by centrifugation, and analyzed for inulin and electrolytes. Glomerular filtration rate (GFR) was equated with the clearance of inulin.

**Targeted tissue low density gene array**

Total RNA was prepared using the Totally RNA kit (Ambion, catalog #AM1910) followed by RNA cleanup using the RNeasy Plus Mini Kit (Qiagen, catalog #74134) according to the manufacturers’ protocols. cDNA was prepared from purified RNA using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor
Quantitative real-time PCR was performed utilizing custom-designed TaqMan® Low Density Arrays (TLDA) from Applied Biosystems. Thermal cycling was performed using an ABI Prism 7900HT FAST Real-time PCR System. Detailed experimental conditions on the TLDA analysis were described previously. The comparative CT method of relative quantification using averaged values for HPRT, PPIA, and GAPDH as normalizers compared to the CT value of the target gene (ΔCT) was used. Relative quantification (RQ, or fold change) between different sample groups was then determined according to the 2−ΔΔCT method, where ΔΔCT = ΔCT treated sample – ΔCT control sample(s). The mean of the expression values for the control+vehicle samples (N=8-10) was used as the calibrator for these calculations.

Analytical methods

Urine and plasma electrolytes were measured by flame-photometer (model 943, Instrumentation Laboratory, Milano, Italy), and inulin concentrations were determined by the Anthrone method. Urine volume was determined gravimetrically. Plasma albumin was determined by the albumin bromcresol-green reaction (Raichem, San Diego, CA, USA). Blood glucose concentrations were measured in tail vein samples by Ascensia Elite blood glucose meter (Bayer plc, Berkshire, UK) and Hematocrit was determined by heparinized capillary tubes (Marienfeld, Lauda-Königshofen, Germany). Serum aldosterone was determined using Coat-A-Count solid-phase 125I RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma insulin was measured by rat insulin RIA kit (Linco Research, St. Charles, MO, USA). Plasma brain natriuretic peptide (BNP)-32 was determined by an
enzyme-linked immunosorbent assay (ELISA) (AssayMax rBNP-32 ELISA kit, Assaypro, St. Charles, MO, USA). Plasma adiponectin was measured using ELISA (Mouse/Rat Adiponectin ELISA kit, B-Bridge International, Mountain View, CA, USA).

**Statistical analysis**

One-way analysis of variance (ANOVA) and two-way ANOVA for repeated measurements were used for group comparison, as appropriate. Tukey’s and Bonferroni’s corrections for multiple comparisons were used as ANOVA post hoc tests, respectively. Repeated measures one-way ANOVA, followed by Dunnett’s multiple comparison test, was used to test significance of change from baseline values of clearance parameters within treatment groups in the volume expansion experiments. P=0.05 was chosen as the significance level for all analyses. Data are expressed as means±SE.
Supplemental references


