Uremic cardiomyopathy is characterized by loss of the cardioprotective effects of insulin

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Emerging feature of UCM is an enhanced susceptibility of the heart to ischemia-reperfusion injury (IRI) (23). IRI is a complex process in which the final common pathway for cellular damage is opening of the mitochondrial permeability transition pore (mPTP) (31), itself the focus of an endogenous protective cascade, termed the reperfusion injury salvage kinase (RISK) pathway (33). Diverse strategies and ligands, including insulin, have been identified which activate this cascade and confer significant cardioprotection in experimental models (33). In addition, small clinical trials and retrospective analyses of larger clinical studies suggest favorable outcomes when RISK-activating interventions are given early (4, 59, 60).

Insulin resistance remains an independent risk factor for cardiac death in CKD stages 3–5 and end-stage renal failure (6, 75, 82). The insulin-signaling cascade converges on the RISK pathway at protein kinase Akt (also known as protein kinase B), and insulin administration is cardioprotective in nonuremic experimental models of IRI (37). Both experimental and clinical CKD have been associated with insulin resistance due to a postreceptor defect in skeletal muscle (46), raising the possibility of impaired activation of the RISK pathway as a mechanism to explain the enhanced susceptibility to IRI. Experimental uremia is associated with increased chronic Akt phosphorylation and activation (49), which have been experimentally demonstrated to inhibit RISK pathway cardioprotection in nonhypertrophied hearts (56).

Thiazolidiones (TZDs) enhance glycemic control in insulin-resistant states, although their widespread clinical use is limited by adverse effects on heart failure (18, 58, 77). However, experimental pretreatment with TZDs offers cardioprotection in models of IRI (48, 89). In particular, rosiglitazone exerts cardioprotective effects in other nonuremic but insulin-resistant states (90), an action mediated, at least in part, through the Akt and the RISK pathway (89), via cardiac peroxisome proliferator-activated receptor-γ (PPARγ) (87).

There is a great clinical need to better understand the pathophysiology of UCM. Although CKD is a chronically progressive condition, the risk of progression to end-stage renal failure is overshadowed at all stages by a greater risk of death from cardiovascular causes (32, 42). This excessive cardiovascular risk continues after the initiation of renal replacement therapy, and half of all deaths in the dialysis population are a result of cardiovascular events (25, 47). Furthermore, in the hemodialysis population, there is increasing evidence that hemodialysis itself results in repeated cardiac events.

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IRI episodes that adversely affect cardiac function and prognosis (11).

To date, there are no data on the functional consequences of IRI, or the efficacy of the cardioprotective RISK pathway in experimental uremia. Using the surgical remnant kidney model of chronic uremia, this study investigated the hypothesis that the myocardial insulin resistance evident in UCM enhances the susceptibility of the uremic heart to ischemic reperfusion injury through impaired response of the RISK pathway. Furthermore, we hypothesized that pretreatment with the insulin-sensitizing agent rosiglitazone would improve cardioprotection in UCM.

METHODS

Experimental Model of Uremia

All animal experiments conformed to the UK Animals (Scientific Procedures) Act (1986) and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). Uremia was induced in male Sprague-Dawley rats (~250 g, Charles River, Sussex, UK) via a one-stage 5/6th nephrectomy as described previously (79). Briefly, animals were anesthetized using a mixture of isoflurane in oxygen (2.5% in 1 liter), a laparotomy was performed, the left kidney was exposed, and at least two-thirds removed. This was immediately followed by a total right nephrectomy. Care was taken to ensure no damage was done to the adrenal glands. For control animals, a sham operation was performed whereby both kidneys were decapsulated and replaced intact.

Animals were maintained for 12 w postinduction of uremia, housed individually, and pair-fed with control animals. Water was available ad libitum. Cardiac hypertrophy was assessed at the time of death by determining wet heart weight/tibia length (HW:TL).

Isolated Heart Perfusion

Animals were fasted for 12 h, anesthetized with sodium thiopentone (100 mg/kg body wt), and the hearts were excised. Hearts were perfused via the aorta in an isovolumic Langendorff mode, as described previously (79), using Krebs-Henseleit buffer containing 3% fatty acid-free BSA and the following components (in mM): 118.5 NaCl, 25 NaHCO3, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.25–2.5 CaCl2, 5 glucose, 0.1 sodium pyruvate, 1 sodium lactate, 0.3 sodium palmitate, and 0.5 glutamine ± 0.1 μM insulin. The buffer was gassed with 95% O2:5% CO2 and maintained at 37°C.

Cardiac function was recorded continuously via a fluid-filled balloon (inserted into the left ventricle) and a physiological pressure transducer (SensNor) connected to a bridge amplifier and Powerlab 4/30 (79). Data were recorded using Chart 5.5 software (AD Instruments, Hastings, UK). The end-diastolic pressure (EDP) was set to 4–30 (79). Data were recorded using Chart 5.5 software (AD Instruments, Hastings, UK). The end-diastolic pressure (EDP) was set to 4–30 (79). Data were recorded using Chart 5.5 software (AD Instruments, Hastings, UK). The end-diastolic pressure (EDP) was set to 4–30 (79). Data were recorded using Chart 5.5 software (AD Instruments, Hastings, UK).

Protein Expression

Expression of total Akt, pAkt, GSK3β, phospho GSK3β (pGSK3β), ANP, and β-actin in uremic and control hearts were determined by Western blotting as described previously (79). Briefly, samples containing 20 μg protein were separated on 10% SDS PAGE and transferred onto nitrocellulose membranes. Membranes were incubated with primary antibodies [rabbit monoclonal Anti-Akt, anti-pAkt (ser473), anti-GSK3β, anti-pGSK3β (ser9), and anti-β-actin at 1:1,000 dilution, New England Biolabs] and rabbit polyclonal anti-ANP (72). Secondary antibody (1:2,000 dilution goat anti-rabbit, Santa Cruz Biotechnology, Santa Cruz, CA) followed by secondary antibody (1:2,000 dilution goat anti-rabbit, Santa Cruz Biotechnology). Protein bands were visualized using enhanced chemiluminescence (ECL; Amersham, Uppsala, Sweden) and quantified using scanning densitometry and ImageJ software. β-Actin was used as the loading control.

Expression of total and phosphorylated Akt1 and Akt2 were determined by first immunoprecipitating the isomer of interest from a crude extract using anti-Akt isomer monoclonal antibodies (1:50, anti-Akt1 and anti-Akt2, New England Biolabs) with agarose beads, before separation of proteins by 10% SDS-PAGE and Western blotting with anti-Akt and anti-pAkt (ser473) as above.
Rosiglitazone Administration

Rosiglitazone (Avandia, GlaxoSmithKline) was administered to animals by oral gavage at a dose of 3 mg·kg⁻¹·day⁻¹ for 8 days before experimentation as described (91). Treatment control animals received identical volumes (by weight) of vehicle (PEG).

Experimental groups were control (C) vs. uremic (U), rosiglitazone untreated (−R) and -treated (+R), and non-insulin (−I) and insulin (+I)-treated, resulting in eight groups in total.

Statistical Analysis

Results are expressed as means ± SE. Statistical significance was determined using an unpaired t-test (for single mean comparisons) or two-way ANOVA for testing two independent variables (using the Scheffé post hoc test). Pearson’s analysis was used to determine the significance of bivariate correlations. Statistical analysis was performed using SPSS software (16.0), and the level of significance was set at P < 0.05.

Table 1. Effect of uremia and rosiglitazone on renal function, anemia, anthropometric measurements, and serum metabolic substrate concentrations

<table>
<thead>
<tr>
<th></th>
<th>C-R (n = 10)</th>
<th>C+R (n = 15)</th>
<th>U-R (n = 10)</th>
<th>U+R (n = 15)</th>
<th>C vs. U</th>
<th>-R vs. +R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain, g</td>
<td>268 ± 13</td>
<td>266 ± 9</td>
<td>266 ± 15</td>
<td>253 ± 12</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Tibia length, cm</td>
<td>4.48 ± 0.03</td>
<td>4.47 ± 0.03</td>
<td>4.51 ± 0.04</td>
<td>4.48 ± 0.02</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.57 ± 0.04</td>
<td>1.56 ± 0.08</td>
<td>1.79 ± 0.08*</td>
<td>1.80 ± 0.53#</td>
<td>&lt;0.05</td>
<td>ns</td>
</tr>
<tr>
<td>HW:TL, g/cm</td>
<td>0.35 ± 0.01</td>
<td>0.35 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.01#</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>% Lung water</td>
<td>75.8 ± 0.3</td>
<td>76.7 ± 0.5</td>
<td>76.4 ± 0.3</td>
<td>76.9 ± 0.1</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>LK weight, g</td>
<td>1.40 ± 0.08</td>
<td>1.46 ± 0.04</td>
<td>1.55 ± 0.08</td>
<td>1.54 ± 0.09</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Liver, g</td>
<td>15.2 ± 1.3</td>
<td>13.1 ± 0.9</td>
<td>14.0 ± 1.1</td>
<td>12.8 ± 0.6</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Urea, mM</td>
<td>4.6 ± 0.5</td>
<td>4.9 ± 0.4</td>
<td>11.5 ± 1.1#</td>
<td>13.3 ± 1.1#</td>
<td>&lt;0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Creatinine, μM</td>
<td>28.9 ± 2.3</td>
<td>29.0 ± 1.1</td>
<td>71.6 ± 6.5#</td>
<td>75.0 ± 9.3#</td>
<td>&lt;0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Hct, %</td>
<td>41 ± 1</td>
<td>41 ± 1</td>
<td>35 ± 1#</td>
<td>35 ± 1#</td>
<td>&lt;0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Glucose, mM*</td>
<td>8.4 ± 0.6</td>
<td>6.2 ± 0.48</td>
<td>7.2 ± 0.5</td>
<td>5.9 ± 0.5</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Insulin, μg/Pb</td>
<td>1.33 ± 0.32</td>
<td>0.89 ± 0.17</td>
<td>1.07 ± 0.13</td>
<td>0.89 ± 0.26</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HOMA-IR, mmol/l × μU/mlb</td>
<td>12.1 ± 2.3</td>
<td>6.4 ± 1.48</td>
<td>8.6 ± 1.5</td>
<td>6.3 ± 2.2</td>
<td>&lt;0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Free fatty acids, mM</td>
<td>0.31 ± 0.04</td>
<td>0.36 ± 0.04</td>
<td>0.32 ± 0.05</td>
<td>0.35 ± 0.05</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SE for serum urea, creatinine, and hematocrit values, anthropometric measures, and serum metabolic substrate concentrations in control and uremic animals. Rosiglitazone had no significant effect on either renal function or cardiac hypertrophy but was associated with lower serum glucose and insulin concentrations, and thus homeostasis model assessment-immunoreactivity (HOMA-IR) values. No change was detected in serum free fatty acid concentrations. HW:TL, wet heart weight/tibia length; ns, Not significant; C-R, control no rosiglitazone; C+R, control plus rosiglitazone; U-R, uremic, no rosiglitazone; U+R, uremic plus rosiglitazone. P values are given for 2-way ANOVA; subgroup analysis was by Scheffé post hoc test. *
P < 0.05 vs. no rosiglitazone; #
P < 0.05 vs. respective control:

Table 2. Effect of uremia and insulin on in vitro baseline cardiac function, efficiency, and metabolism in control and uremic hearts

<table>
<thead>
<tr>
<th></th>
<th>C-I (n = 8)</th>
<th>U-I (n = 8)</th>
<th>C+I (n = 6)</th>
<th>U+I (n = 8)</th>
<th>C vs. U</th>
<th>-I vs. +I</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPP, ×10³ mmHg/min</td>
<td>45 ± 3</td>
<td>49 ± 2</td>
<td>45 ± 5</td>
<td>51 ± 3</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>dP/dmax, mmHg/s</td>
<td>4.480 ± 756</td>
<td>5.426 ± 561</td>
<td>5.960 ± 400</td>
<td>6.579 ± 368</td>
<td>ns</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>dP/dmin, mmHg/s</td>
<td>−2.929 ± 160</td>
<td>−3.311 ± 142</td>
<td>−3.127 ± 216</td>
<td>−3.566 ± 193</td>
<td>ns</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MVO₂, μmol·g⁻¹·min⁻¹</td>
<td>0.85 ± 0.06</td>
<td>0.82 ± 0.06</td>
<td>0.65 ± 0.10</td>
<td>0.65 ± 0.06</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Efficiency, ×10³ mmHg·μmol⁻¹·g wet wt⁻¹</td>
<td>5.4 ± 0.4</td>
<td>6.4 ± 0.07</td>
<td>7.6 ± 1.0</td>
<td>8.3 ± 0.9</td>
<td>ns</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glucose, %</td>
<td>10.6 ± 1.2</td>
<td>12.9 ± 1.8</td>
<td>12.3 ± 0.3</td>
<td>17.3 ± 1.4#</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Palmitate, %</td>
<td>48.8 ± 2.1</td>
<td>37.8 ± 2.2#</td>
<td>31.4 ± 1.8#</td>
<td>16.1 ± 0.9#</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Uateral, %</td>
<td>40.6 ± 1.9</td>
<td>49.3 ± 2.2#</td>
<td>56.3 ± 1.9#</td>
<td>66.6 ± 1.9#</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE for measures of cardiac function and relative substrate contribution of acetyl-CoA to the Krebs cycle in control and uremic hearts perfused in the absence or presence of insulin. RPP, rate pressure product; MVO₂, myocardial oxygen consumption. Uremia was not associated with a decline in measures of cardiac function. Insulin acted to increase cardiac contractility (dP/dt) and efficiency independently of uremia. Uremia was associated with a significant shift from fatty acid to carbohydrate metabolism, yet substrate selection remained sensitive to insulin. C-I, control no insulin; U-I, uremic no insulin; C+I, control plus insulin; U+I, uremic plus insulin. P values are given for 2-way ANOVA, subgroup analysis was by Scheffé post hoc test. *P < 0.05 vs. no insulin. # P < 0.05 vs. respective control.
The effects of IRI were assessed during ex vivo cardiac perfusion. Twenty-five minutes of total global ischemia produced a significantly greater impairment of cardiac function in uremic hearts [max recovered RPP (%) 59.6 ± 10.7 vs. 19.3 ± 4.6, n = 10, P < 0.05; Fig. 1] than in controls. Furthermore, while insulin demonstrated a cardioprotective effect in control hearts [max recovered RPP (%) 59.6 ± 10.7 vs. 88.9 ± 8.5, n = 10, P < 0.05], consistent with other published studies (29, 37, 39, 52, 92), such an effect was not seen in uremic hearts [max recovered RPP (%) 19.3 ± 4.6 vs. 28.5 ± 10.4, n = 10, P = ns; Fig. 1].

Rosiglitazone Therapy is Associated with Restoration of Cardioprotective Effects of Insulin in the Uremic Heart

The ability of the oral TZD rosiglitazone to resensitize the uremic heart to the protective effects of insulin was investigated in the uremic model. Administration of rosiglitazone at a dose of 3 mg·kg⁻¹·day⁻¹ for 8 days had no effect on weight gain, tibia length, renal function, or anemia in either group (Table 1). Nor did it affect hypertrophy of the remnant kidney or heart in uremic animals. Overall, rosiglitazone reduced fasting glucose and insulin concentrations, and thus HOMA-IR (Table 1), although this effect did not reach statistical significance in uremic animals. Rosiglitazone had little effect on serum fatty acid levels. Baseline ex vivo cardiac function was also unaffected by rosiglitazone administration (Table 3).

In control hearts, rosiglitazone appeared to improve post-IRI function but did not modify the cardioprotective effect of insulin (Fig. 2 and Table 4). In uremic hearts, however, an overall positive effect on cardiac recovery was associated with rosiglitazone treatment (Fig. 3 and Table 5). The combination of rosiglitazone and insulin treatment was also linked with significant improvements in recovery of cardiac function, as evidenced by increased RPP, dP/dt max, and dP/dt min, and was associated with greater recovery than either treatment alone.

Uremia is Associated with Altered Activation of the Common Insulin-Signaling and RISK Pathway Intermediate Akt but Not the Common Intermediate GSK3β

Protein expression in ventricular muscle (before ischemia) of total Akt, phospho Akt, Akt1 and Akt2 isoforms, total GSK3β and phospho GSK3β was assessed by immunoblotting and immunoprecipitation. Ventricular muscle phospho Akt was significantly increased in the uremic hearts (relative optical den-

Table 3. Effect of rosiglitazone on in vitro baseline cardiac function

<table>
<thead>
<tr>
<th></th>
<th>C-R (n = 9)</th>
<th>C+R (n = 10)</th>
<th>U-R (n = 9)</th>
<th>U+R (n = 10)</th>
<th>C vs. U</th>
<th>R vs. +R</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPP, ×10⁴ mmHg/min</td>
<td>30.1 ± 1.9</td>
<td>28.1 ± 1.7</td>
<td>32.5 ± 1.4</td>
<td>29.1 ± 1.5</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>3,371 ± 217</td>
<td>3,122 ± 238</td>
<td>3,402 ± 234</td>
<td>3,074 ± 204</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>dP/dt min, mmHg/s</td>
<td>−1,926 ± 87</td>
<td>−1,903 ± 101</td>
<td>−2,002 ± 81</td>
<td>−1,856 ± 66</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>MVO₂, μmol·g⁻¹·min⁻¹</td>
<td>0.93 ± 0.05</td>
<td>0.95 ± 0.03</td>
<td>0.98 ± 0.03</td>
<td>0.93 ± 0.04</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Efficiency, ×10⁻¹ mmHg·μmol⁻¹·g wet wt⁻¹</td>
<td>32.1 ± 2.4</td>
<td>29.9 ± 1.8</td>
<td>33.8 ± 1.8</td>
<td>31.6 ± 1.8</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SE for RPP, contractility (dP/dt max), relaxation (dP/dt min), MVO₂, and efficiency in control and uremic animals ± rosiglitazone therapy before ischemia-reperfusion. Groups are defined as in Table 1. P values are given for 2-way ANOVA; subgroup analysis was by Scheffé post hoc test. a, C-R, n = 8; C+R, n = 10; U-R, n = 6; U+R, n = 10.
sity 1.00 ± 0.08 vs. 1.3 ± 0.11; n = 12, P < 0.05; Fig. 4), which was predominantly attributable to phosphorylation of Akt2 rather than Akt1 (Fig. 5). However, there was no change in phospho and total GSK3β associated with uremia (pGSK3β 1.00 ± 0.05 vs. 0.90 ± 0.03, n = 23, P = ns; total GSK3β 1.00 ± 0.03 vs. 0.88 ± 0.06, n = 23, P = ns).

The presence of insulin at reperfusion was associated with an increase in both phospho Akt and phospho GSK3β independently of uremia (control: pAkt 1.00 ± 0.6 vs. 17.4 ± 3.5, n = 12, P < 0.05; pGSK3β 1.0 ± 0.1 vs. 2.5 ± 0.3, n = 12, P = ns; uremic: pAkt 3.5 ± 1.8 vs. 14.5 ± 7.1, n = 12, P < 0.05; pGSK3β 1.0 ± 0.2 vs. 4.7 ± 2.2, n = 12, P < 0.05). Uremia was not related to a change in postischemic Akt1 phosphorylation or expression, but both insulin and uremia produced independent reductions in total Akt2 (tAkt2) expression post-IRI (Fig. 6).

DISCUSSION

UCM Is Characterized by Increased Susceptibility to IRI and a Failure of Insulin-Mediated Cardioprotection: Potential Role for Altered RISK Pathway Activation

Uremic hearts displayed significantly reduced functional recovery during reperfusion (Fig. 1). These observations complement those of Dikow et al. (22, 23), who demonstrated increased infarct size following temporary occlusion of the left coronary artery in uremic rats in vivo and are consistent with the clinical picture of adverse outcomes in patients with IHD and CKD (76, 86). One other study by Raine et al. (64) investigated the susceptibility of the uremic heart to IRI and observed increased inosine release, a measure of ATP catabolism and thus indicative of enhanced myocyte damage. In the study presented here, total global ischemia in an ex vivo setting has been employed to assess ischemic injury. The consistent observation of enhanced susceptibility of the uremic heart to IRI under these in vitro conditions confirms that this is a function of UCM, rather than purely a consequence of the in vivo milieu.

The continuing responsiveness of cardiac metabolism to insulin in uremia during in vitro perfusion (Table 2) indicates that the increased susceptibility to IRI here is not a result of reduced metabolic flexibility (2, 26). However, the lack of the cardioprotective effect of insulin (Fig. 1) and the alterations in Akt phosphorylation in uremic hearts (Figs. 5 and 6) demonstrated in this study indicate that an underlying defect in the RISK pathway is more likely responsible.

The lack of insulin-mediated cardioprotection in uremic hearts is in contrast to experimental studies in normal hearts and nonuremic models of cardiac hypertrophy (37, 67). This

**Table 4. Effect of insulin and rosiglitazone on maximal recovery of control hearts after ischemia-reperfusion injury**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-I-R (n = 4)</th>
<th>C+I-R (n = 5)</th>
<th>C-I+R (n = 5)</th>
<th>C+I+R (n = 5)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPP, % recovery</td>
<td>35.5 ± 10.4</td>
<td>85.9 ± 14.4</td>
<td>57.0 ± 19.5</td>
<td>90.0 ± 17.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>dP/dtmax, % recovery</td>
<td>34.4 ± 8.1</td>
<td>65.9 ± 24.5</td>
<td>55.9 ± 19.2</td>
<td>87.7 ± 17.8</td>
<td>ns</td>
</tr>
<tr>
<td>dP/dtmin, % recovery</td>
<td>70.0 ± 20.8</td>
<td>79.1 ± 24.6</td>
<td>65.6 ± 23.4</td>
<td>93.2 ± 20.5</td>
<td>ns</td>
</tr>
<tr>
<td>MVO2, μmol·g⁻¹·min⁻¹</td>
<td>0.91 ± 0.01</td>
<td>0.94 ± 0.08</td>
<td>0.98 ± 0.03</td>
<td>0.89 ± 0.04</td>
<td>ns</td>
</tr>
<tr>
<td>Cardiac efficiency, % recovery</td>
<td>36.4 ± 12.4</td>
<td>82.3 ± 12.3</td>
<td>57.1 ± 19.6</td>
<td>83.0 ± 17.1</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE of maximal % recovery of baseline cardiac function in control hearts after 25-min warm total global ischemia. Groups are defined in Tables 1–3. *P < 0.05 -I-R vs. +I-R. §P < 0.05 -I+R vs. +I+R.

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therefore may be a unique finding of UCM. However, Dikow et al. (23) demonstrated a reduction in infarct size in uremic hearts exposed to hyperinsulinemic euglycemic clamping (for 45 min before ligation of the left anterior descending coronary artery and continued throughout ischemia and reperfusion). Two significant differences in study design may account for the apparent contradiction here. First, Dikow et al. administered insulin before the ischemic insult, in effect a preconditioning stimulus. The protective effects of preconditioning and postconditioning may be linked by the RISK pathway (33). However, neither process is fully characterized and it is possible for aspects of the preconditioning pathway to remain effective while the postconditioning pathway is not. Second, the concurrent administration of a significant glucose load can enhance myocardial glucose metabolism, an intervention known to be cardioprotective (50). However, both of these potential mechanisms should have improved the outcome in the control group, which was not the case, suggesting that in Dikow’s study (23) cardioprotection was not conferred through previously identified mechanisms such as the RISK pathway.

The cardioprotective effects of insulin have been widely studied utilizing a range of cardiac preparations (29, 39, 52, 92). Insulin has been shown to reduce cell death and improve function during the reperfusion period. These effects are critically dependent on both Akt and GSK3β phosphorylation (33, 40). The loss of insulin-mediated cardioprotection observed in the uremic heart may therefore reflect impaired signaling through these key components of the RISK pathway. In the absence of IRI, uremia was associated with increased phospho Akt expression (Fig. 4). While this might be predicted to be cardioprotective, the findings of Nagoshi et al. (56) suggest that chronic activation of the RISK pathway can also lead to downregulation of key intermediates and a loss of the cardioprotective phenotype. While the data presented here do not show a deficit in insulin-stimulated phosphorylation of Akt or GSK3β in unfractionated cellular extracts, closer investigation of Akt isoform expression post-IRI suggests alterations in Akt2 expression (Fig. 6). DeBosch et al. (21) have previously demonstrated that Akt 2 rather than Akt1 underlies RISK-mediated cardioprotection. Data here support the concept that insulin stimulation alters Akt2 expression post-IRI in control and uremic hearts, with an additional independent reduction in post-IRI levels of Akt2 in uremic hearts. Thus the Akt signaling axis is modified in the posts ischemic uremic heart, raising the possibility of its involvement in the increased IRI susceptibility.

Table 5. Effect of insulin and rosiglitazone on maximal recovery of uremic hearts after ischemia-reperfusion injury

<table>
<thead>
<tr>
<th></th>
<th>U-I-R (n = 4)</th>
<th>U-I+R (n = 5)</th>
<th>U-I-R (n = 5)</th>
<th>U-I+R (n = 5)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPP, % recovery</td>
<td>11.5 ± 6.2</td>
<td>12.7 ± 7.0</td>
<td>46.2 ± 19.2</td>
<td>61.8 ± 15.9#</td>
<td>ns</td>
</tr>
<tr>
<td>dP/dtmax, % recovery</td>
<td>22.0 ± 5.8</td>
<td>42.5 ± 17.1</td>
<td>31.0 ± 25.6</td>
<td>62.1 ± 16.9#</td>
<td>ns</td>
</tr>
<tr>
<td>Cardiac efficiency, % recovery</td>
<td>15.8 ± 9.4</td>
<td>15.8 ± 8.8</td>
<td>47.3 ± 19</td>
<td>65.2 ± 15.4#</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SE of maximal % recovery of baseline cardiac function in uremic hearts after 25-min warm total global ischemia. Groups are defined as in Tables 1–3.
Rosiglitazone Therapy Resensitized the Uremic Heart to Cardioprotective Effects of Insulin

In uremic hearts, rosiglitazone treatment improved functional recovery (RPP) at all time points in addition to all measures of function and efficiency. The addition of insulin post-IRI to rosiglitazone-treated hearts had an additive effect, achieving significant increases in RPP at all time points (Fig. 3 and Table 5). These results demonstrated that, despite the lack of effect of insulin alone, IRI damage in the uremic heart is amenable to salvage by selected interventions, and further that rosiglitazone treatment is capable of “resensitizing” the uremic heart to the prosurvival effects of insulin. Rosiglitazone demonstrated modest prosurvival effects in control hearts in keeping with previous studies (54) but did not provide additional benefit to insulin treatment alone (Fig. 2 and Table 4). This may represent a maximal effect as control hearts exposed to insulin at reperfusion are already achieving a recovery of RPP of ~85%.

These results complement those of Taniguchi et al. (83), who have demonstrated pioglitazone-mediated IRI cardioprotection in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a nonuremic model of insulin resistance. They also identified pioglitazone-mediated enhancement of stress-induced Akt phosphorylation, suggesting that this is the likely path of action. The resensitization to the effects of insulin in the study presented here also suggests that rosiglitazone is acting through the RISK-Akt pathway as has been demonstrated in other experimental models utilizing both rosiglitazone and the related TZD pioglitazone. Yue et al. (91), utilizing diabetic rats and rosiglitazone administration as in this study, demonstrated rosiglitazone treatment conferred a similar degree of cardioprotection, with enhanced post-IRI Akt phosphorylation. Cardioprotection and Akt phosphorylation were almost completely abolished by inhibition of the upstream RISK pathway intermediate phosphatidylinositol 3-kinase (PI3K), with Wortmannin. Liu et al. (48) demonstrated in hypercholesterolemic rabbit hearts rosiglitazone-mediated reductions in post-IRI apoptosis, a well-recognized effect of Akt activation, although that was not directly assessed in their study. Cao et al. (16) confirmed pioglitazone-mediated reductions in post-IRI cardiomyocyte apoptosis in the rat heart associated with reduced caspase 3 and Bax expression (proapoptotic) and increased Bcl-2 expression (antiapoptotic), alterations normally associated with Akt activation (38, 63, 88).

More recently, Yasuda et al. (89) have demonstrated pioglitazone-mediated protection against in vivo myocardial infarction of rabbit hearts linked to increased phospho Akt and phospho endothelial nitric oxide (NO) synthase (eNOS). eNOS is a downstream target of Akt (28, 52, 81), and Akt-mediated prosurvival effects are dependent on generation of NO. (28) Protection was abrogated in Yasuda’s study by the application of inhibitors of PPARγ, PI3K, and NOS inhibition, providing strong evidence for a TZD-PI3K-Akt-eNOS-mediated mechanism of cardioprotection post-IRI.

There is a great clinical need to modify the functional consequences of UCM. In the hemodialysis population, particular strategies to improve the tolerance of the heart to ischemic insult are urgently required. McIntyre et al. (13, 14, 20) have assessed cardiac function and damage during hemodialysis
using serum troponin T concentrations, serial echocardiography, and serial positron emission tomography scans. They have demonstrated repeated episodes of myocardial ischemia (myocardial stunning) and regional ventricular dysfunction associated with hemodialysis and furthermore that the presence of such defects predicts deterioration of cardiac function in the subsequent 12 mo (13). As hemodialysis is a predictable event, it is amenable to prophylactic strategies, avoiding the pitfall of interventions for acute cardiac ischemia when the protective intervention is often administered too late (4). Pretreatment-protective interventions have already been successful in other clinical situations, such as coronary artery bypass grafting and acute myocardial infarction (AMI) (9, 35, 44, 60).

Clinically, rosiglitazone treatment has been associated with exacerbation of heart failure and an enhanced incidence of myocardial infarction in non-CKD populations (10, 32, 42). However, as discussed above, pioglitazone also has experimental data to support a role in activation of the RISK pathway. Furthermore, there is no excess risk of myocardial infarction or heart failure associated with its use in clinical practice (30). There is also theoretical reason to suspect clinically significant differences in outcomes in the end-stage renal failure population, where the effects of rosiglitazone on the distal tubule (41) will be diminished. The safety of TZDs in renal failure has been tested in a number of post hoc or retrospective studies. Schneider et al. (70) performed a post hoc analysis of the PROactive trial and demonstrated reduced all-cause mortality, myocardial infarction, and stroke in patients with CKD (glomerular filtration rate <60 ml-min⁻¹·1.73m⁻²) treated with pioglitazone. Subsequently, Ramirez et al. (65) demonstrated increased all-cause mortality in rosiglitazone-treated patients on hemodialysis enrolled in the Dialysis Outcomes and Practice Patterns Study (DOPPS). However, Brunelli et al. (12) have shown reduced all-cause mortality for hemodialysis patients receiving either pioglitazone or rosiglitazone, with no significant difference between the two agents. The safety of TZDs in CKD therefore remains unclear (8). Rosiglitazone treatment in this study was utilized in a way previously shown to be cardioprotective in experimental IRI (91). The duration of treatment was too short to have a significant effect on cardiac hypertrophy. However, a trend toward reduced cardiac function and a statistically significant increase in percent lung water were noted (Tables 1 and 3).

These findings and the clinical data limit the direct “translatability” of the positive findings in this study. However, the core finding of successful improvement in functional outcomes in uremic hearts after IRI should provoke further investigation of alternative protective strategies involving the Akt-eNOS pathway. Many such alternatives have been investigated in nonuremic models (7, 33). Disappointingly, the extensive preclinical data are not yet matched by corresponding success in clinical trials. Adenosine or the synthetic adenosine receptor agonist AmP579 have been examined in three clinical trials (45, 53, 68), the results of which are mixed and essentially flawed by aspects of the study design (59). However, complete reanalysis of the largest of these trials, AMISTAD-II, has shown benefit (reduced early and late survival, and reduced death or heart failure composite endpoint at 6 mo) to the use of adenosine as an adjunct to reperfusion in acute myocardial infarction in those with a short duration of ischemic symptoms (44).

Another alternative mechanism of RISK pathway activation with early positive outcomes in human studies is “ischemic postconditioning,” an extension of the original discovery by Murry et al. (55) of reduced IRI after repeated episodes of brief ischemia before the index ischemic event (ischemic preconditioning). Ischemic postconditioning was first defined by Kin et al. (43), and the mechanism has since been extensively studied and found to involve activation of the RISK pathway (78). Since 2005, there have been several translational clinical studies demonstrating significant reductions in infarct size and improved functional parameters when ischemic postconditioning is utilized in the treatment of AMI (19, 80, 84). However, the general utility of this method is limited by the need for access to the coronary circulation. More attractive might be the concept of remote ischemic conditioning, in which repeated brief ischemia to an organ remote from the heart, either preischemia or prererefuson, confers protection from IRI (34, 62). Two human studies, one in human volunteers and patients with coronary artery disease (51) and one in the setting of AMI (9) have shown that repeated brief (5 min) limb ischemia, induced using an inflatable cuff, improves endothelial function and reduces infarct size following reperfusion. Furthermore,
investigation is required to confirm these results, but the technique is attractive in instances of predictable IRI, such as hemodialysis.

In an alternative approach, significant cardioprotection in experimental and human studies has been demonstrated through inhibition of the final end effector of IRI, the mPTP (5). This large nonselective pore forms in the mitochondrial inner membrane during reperfusion, resulting in cell death through dissipation of the mitochondrial membrane potential, inhibition of ATP production, release of proapoptotic ligands, and swelling and rupture of mitochondria. Inhibition of the opening of the mPTP is the primary effect through which RISK pathway activation reduces IRI (17, 36). The long-established immunosuppressive drug cyclosporin A directly inhibits opening of the mPTP and has previously been shown to be cardioprotective in experimental models (73). Recently, however, a small-scale study has confirmed this effect in humans. Piot et al. (60) demonstrated reduced infarct size with the use of a single bolus of cyclosporin before reperfusion in 58 patients undergoing primary percutaneous coronary intervention for AMI.

Therefore, although TZDs may not be utilized in clinical practice as cardioprotective agents in renal failure, multiple other RISK pathway-activating maneuvers have been identified in experimental studies, and there is a growing body of clinical evidence for their utility in the nonuremic population. Such avenues should be the focus of future studies in the CKD population.

Reduction in HOMA-IR Scores by Rosiglitazone was Reduced in Uremic Animals

Overall, rosiglitazone improved insulin sensitivity as evidenced by reduce HOMA-IR scores (Table 1). However, while exhibiting the same trend, this effect was not statistically significant in subgroup analysis of uremic animals. TZD treatment has previously been reported to have no effect on serum glucose and insulin concentrations in nondiabetic rats (74). However, rosiglitazone did significantly reduce HOMA-IR values in control animals in this model. The lack of statistically significant effect in the uremic group appears to stem from a dilution of the effect due to a additional nonsignificant trend for lower HOMA-IR values in uremic animals.

Metabolic and Pleiotropic Effects of Insulin can be Altered Independently in CKD

Insulin resistance, as it is typically attributed in clinical and experimental studies, relates specifically to one of insulin’s many effects, namely that of serum glucose control. Using this definition, insulin resistance, which remains an independent risk factor for cardiovascular death in CKD (6, 75), has been implicated in the pathogenesis of pathological cardiac hypertrophy (24, 69). However, the data presented here clearly reveal resistance to the pleiotropic effects of insulin, in the absence of systemic metabolic insulin resistance. This is consistent with data from Potenza et al. (61), who have demonstrated defects in discrete pathways of the insulin signaling cascade which leave signal transduction unaffected through other routes.

Resistance to the metabolic effects of insulin results in hyperinsulinemia and imbalance in the various pleiotropic effects of insulin at the level of the endothelium, gene transcription, and protein synthesis that favor cardiac hypertrophy (24, 69). In particular, it appears that hyperinsulinemia can exacerbate pathological cardiac hypertrophy in the presence of other factors. This has been demonstrated in aortic-banded rats (24), where the combination of hyperinsulinemia and hypertension produced significantly more cardiac hypertrophy than hypertension alone. Here, we demonstrate that uremia is associated with a “primary” defect in one of the pleiotropic effects of insulin, independently of metabolic insulin resistance.

Conclusions

This is the first study to demonstrate significantly reduced function of the ex vivo uremic heart after IRI. Furthermore, the loss of insulin-mediated cardioprotection and alterations in Akt expression and phosphorylation suggest an underlying deficit in the RISK pathway.

The insulin-sensitizing agent rosiglitazone restored the cardioprotective effects of insulin, in the uremic heart. Enhanced IRI damage and pathological cardiac hypertrophy occurred in the absence of either systemic or cardiac “metabolic” insulin resistance, despite the utility of insulin resistance as a risk factor for cardiovascular disease in the CKD.

The role of the RISK pathway in the development of UCM and the cardioprotective potential of its manipulation warrant further investigation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.J.S., S.B., and A.-M.L.S. provided conception and design of research; D.J.S. performed experiments; D.J.S. analyzed data; D.J.S. drafted manuscript; D.J.S., S.B., and A.-M.L.S. edited and revised manuscript; D.J.S., S.B., and A.-M.L.S. approved final version of manuscript.

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