Dipeptidyl peptidase-4 inhibition improves left ventricular function in chronic kidney disease

Abstract

Purpose: Heart failure with preserved ejection fraction (HFpEF) is a common comorbidity in people with chronic kidney disease (CKD) for which no evidence-based treatment currently exists. Recently, a group of anti-hyperglycemic agents used in the treatment of Type 2 diabetes, termed incretin-based therapies, have come under scrutiny for their putative glucose-independent effects on cardiac function. In the present study, the actions of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of incretin-based therapy in preventing HFpEF induced by chronic renal impairment were investigated.

Methods: Sham-operated and subtotaly-nephrectomized rats were randomized to receive the DPP-4 inhibitors, linagliptin or sitagliptin for seven weeks before assessment of cardiac and renal structure and function.

Results: Analysis of pressure-volume loops revealed that both linagliptin and sitagliptin prevented the development of cardiac diastolic dysfunction, with cardiac collagen I synthesis also being reduced by DPP-4 inhibition. These attenuating cardiac effects occurred without change in renal function or structure where, in the doses administered, neither linagliptin nor sitagliptin affected GFR decline, proteinuria, renal fibrosis or the increased urinary excretion of biomarkers of renal toxicity.

Conclusion: The beneficial cardiac effects of DPP-4 inhibition, in the absence of a concurrent improvement in renal dysfunction, raise the possibility that these agents may confer cardiovascular advantages in the CKD population.
Although salvage of the native kidney may not be a tangible goal for people with chronic kidney disease (CKD) and advancing severe renal impairment or end-stage renal disease (ESRD), concurrent comorbidities may remain amenable to therapeutic interventions. Among these comorbidities, heart failure with preserved ejection fraction (HFpEF) is particularly common [1], where it carries a mortality risk equivalent to that of systolic heart failure [2], but has no evidence-based treatment. Recently, a new group of agents, termed incretin-based therapies, have come under scrutiny for their potential role in cardiorenal disease. These therapies have been widely adopted into the clinic for their blood glucose lowering properties in individuals with Type 2 diabetes, where they carry a low risk of hypoglycemia.

Incretin-based therapies work primarily by augmenting the actions of the gut hormone, glucagon-like peptide-1 (GLP-1), which functions to stimulate glucose-dependent insulin release and suppress glucagon production [3]. One strategy to augment the actions of GLP-1 is to inhibit its enzymatic degradation by the serine exopeptidase, dipeptidyl peptidase-4 (DPP-4) [4]. GLP-1 acts mainly by binding to its receptor GLP-1R that, despite its primary role in carbohydrate homeostasis, is surprisingly widely distributed. This suggests that the GLP-1R may have a capacity for a much broader mechanism of action [5]. Recent work, including that from our own group, has suggested that DPP-4 inhibitors may improve cardiac diastolic dysfunction by augmenting the actions of both GLP-1 and alternative substrates known to be enzymatically cleaved by DPP-4 [6, 7]. The therapeutic effect of DPP-4 inhibition in HFpEF that accompanies CKD has not, however, been previously evaluated.

In light of a) the common coexistence of HFpEF in the CKD population, b) the urgent need for novel treatments for HFpEF and c) the relative difficulty in dissecting out the tissuespecific effects of DPP-4 inhibition from global improvements in the diabetic milieu, the effect of DPP-4 inhibition was examined in a rodent model of non-diabetic CKD that simultaneously develops HFpEF [8]. Most DPP-4 inhibitors (e.g., sitagliptin, saxagliptin, alogliptin and vildagliptin) are cleared in the urine and require dose adjustment in the CKD population, whereas the DPP-4 inhibitor linagliptin is secreted via the bile [9] and does not require adjustment of dose with declining GFR [10]. Thus, to enhance the clinical applicability of our findings, the effect of two DPP-4 inhibitors, linagliptin and sitagliptin, which are differentiated by their mechanism of elimination, were examined.

**Methods**

**Animals**

Female Fischer rats (344 rats; F344, Charles River, Montreal, Quebec), aged 12 weeks, underwent sham or subtotal nephrectomy surgery as previously described [11, 12]. Ten days post-surgery, rats were randomized to receive either vehicle (0.5% aqueous hydroxyethylcellulose, Sigma-Aldrich, Oakville, ON, Canada), 1.5 mg/kg/day linagliptin (in 0.5% aqueous hydroxyethylcellulose, Boehringer Ingelheim, Ingelheim am Rhein, Germany) or 20 mg/kg/day sitagliptin (in 0.25% carboxymethylcellulose, Sequoia Research Products, Pangbourne, UK) by daily oral gavage for seven weeks (sham + vehicle n=12, subtotally-nephrectomized [SNx] rats + vehicle n=11, sham + linagliptin n=11, SNx + linagliptin n=9, sham + sitagliptin n=14, SNx + sitagliptin n=8). The linagliptin dosage of 1.5 mg/kg is approximately equivalent to 3.2 µmol/kg; midway between two previous doses of the DPP-4 inhibitor employed in an earlier short-term study in SNx rats [13]. All rats were allowed free access to drinking water and standard rat chow ad libitum.

**Metabolic parameters**

Blood glucose was determined using Accu-check Advantage (Roche, Mississauga, ON, Canada). HbA1c was measured using AlcNow+ (Bayer, Sunnyvale, CA, USA). Active GLP-1 was determined using active GLP-1 (v2) kit (Meso Scale Discovery, Rockville, MD, USA).

**Cardiac function**

Transmural echocardiography was performed in anesthetized rats (1% isoflurane) using a Sonos 5500 echocardiograph (Philips Healthcare, Andover, MA, USA) with a high-frequency (5-12 MHz broad-bandwidth) phased array transducer (S12, Philips). Fractional shortening (FS%) was calculated according to the formula: FS% = (LVIDd – LVIDs) / LVIDd X 100, where LVIDd and LVIDs are end-diastolic diameter and end-systolic diameter, respectively, as previously described [14]. Left ventricular mass was derived as previously reported [15]. Three consecutive cardiac cycles were averaged for all analyses.

Cardiac catheterization was performed as previously published [16]. Briefly, rats were anesthetized (2% isoflurane), placed on a warming pad (37°C), intubated and ventilated using positive pressure. The right internal carotid artery was ligated cranially and a 2F miniaturized combined conductance catheter-micromanometer (Model SPR-838, Millar Instruments, Houston, TX, USA) was inserted into the carotid ar-
tery and advanced into the left ventricle until stable pressure volume (PV) loops were obtained [17]. The abdomen was opened and elastic bands were placed around the inferior vena cava and portal vein. All PV loops were obtained with the ventilator turned off for 5-10 seconds and the animal apneic. Data were acquired under steady state conditions and during preload reduction with parallel conductance values obtained by injection of approximately 200 µl 10% NaCl into the right atrium [18, 19]. Calibration from Relative Volume Units (RVU) conductance signal to absolute volumes (in µl) was undertaken using a previously validated method of comparison to known volumes in Perspex wells [20]. A range of functional parameters was then calculated using the pressure conductance data (Millar analysis software PVAN 3.4).

**Renal function**

Urine protein excretion was determined after housing rats in metabolic cages for 24 h. Urinary excretion of markers of renal injury was determined using Rat KidneyMAP v1.0 (Myriad RBM, Austin, TX, USA) and expressed as the ratio to urine creatinine determined by autoanalyzer. Glomerular filtration rate (GFR) was determined by FITC-inulin clearance, as previously described [11].

**Tissue collection**

Rats were anesthetized (2.5% isoflurane) before cervical dislocation. Cardiac tissue was either fixed in 10% neutral buffered formalin (NBF) or flash frozen in liquid nitrogen before storage at -80°C for future molecular biological analysis. The left renal artery was clamped and the kidney was removed, weighed, sliced transversely and immersed in 10% NBF for 24 h. Formalin-fixed tissues were routinely processed, embedded in paraffin and sectioned.

All experimental procedures adhered to the guidelines of the Canadian Council on Animal Care and were approved by the St. Michael's Hospital Animal Care Committee.

**Plasma DPP-4 activity**

DPP-4 activity was determined in frozen plasma samples from rats treated with vehicle, linagliptin or sitagliptin for four days, 1 h and 24 h post-gavage (n=3/group), as previously described [13].

**Cardiac structure**

**Cardiac gene expression**

SYBR green based real time PCR was performed on an Applied Biosystems, Foster City, CA, USA using the following primer sequences: collagen I forward TGCCGATGTCGCTATCCA, reverse TCTT GCAGTGATAGGTGATTTCTG; α-myosin heavy chain (MHC) forward TGTGAAAAGATTTACCGAGTTTAAG, reverse TCTGACTCTCGGAGGTATCG; β-MHC forward GTGCCAAAGGCGCTGAATGAG, reverse GCAA AGGCTCCAGGCTCTGA; atrial natriuretic peptide (ANP) forward ATGGGCTCCTTCTCCATCAC, reverse TCTTC GGATCGGAACGCT; RPL13a forward GATGAACACCA ACCCGTCCTC, reverse CACCATCCGTCTTTTCTGTC. Brain natriuretic peptide (BNP) primers were from Qiagen (Germantown, MD, USA). Data analysis was performed using Applied Biosystems Comparative CT method.

**Immunohistochemistry for cardiac collagen I**

Immunohistochemistry for fibrillar collagen type I (Southern Biotech, Birmingham, AL, USA) was performed as previously described [21] with the antibody used at a concentration of 1:400. Stained heart sections were scanned with the Aperio ScanScope system (Aperio, Vista, CA, USA) and analysed with ImageScope (Aperio). Interstitial collagen I was determined as the proportional area of positive immunostaining in 10 randomly selected fields (x100 magnification).

**Myocyte hypertrophy**

Cardiac myocyte hypertrophy was determined on haematoxylin and eosin (H&E) stained sections as previously reported [17, 22].

**Renal structure**

**Glomerulosclerosis index**

A minimum of 50 glomeruli were examined in PAS-stained kidney sections from each rat. The degree of sclerosis was subjectively graded on a scale of 0 to 4, in a masked manner, as previously described [23]. A glomerulosclerosis index (GSI) was calculated as the mean of the scores obtained by two independent observers.
Immunohistochemistry for glomerular capillary density and tubulointerstitial collagen IV

Immunohistochemistry of kidney tissue was performed as previously described [23-25] with antibodies in the following concentrations: JG-12 1:1000 (Bender Medsystems, Vienna, Austria) and collagen IV 1:100 (Southern Biotech). Kidney sections were scanned with the Aperio ScanScope system and analysed with ImageScope. Glomerular endothelial (JG-12) immunostaining was determined in 30 glomerular profiles from each rat kidney section [23, 26]. For estimation of tubulointerstitial collagen IV, the proportional area of positive immunostaining (excluding glomeruli) was determined in 10 randomly-selected cortical fields (x100 magnification).

Statistics

Data are expressed as means ± SEM except numerical proteinuria data that are presented as median (range). Statistical significance was determined by one-way ANOVA with a Newman-Keuls post-hoc comparison. Skew distributed urinary data were log-transformed before comparison. All statistical analyses were performed using GraphPad Prism 6 for Mac OS X (GraphPad Software, San Diego, CA, USA).

Results

Effects of subtotal nephrectomy and DPP-4 inhibition on plasma DPP-4 activity

In initial experiments, the effects of different doses of linagliptin and sitagliptin on plasma DPP-4 activity were compared in sham-operated and subtotally-nephrectomized (SNx) rats. Since it is renally excreted, sitagliptin was “dose-adjusted” to achieve a magnitude of DPP-4 inhibition equivalent to that observed with linagliptin in SNx rats with renal impairment. Animals were therefore treated with either vehicle, 1.5 mg/kg/day linagliptin, or 20 mg/kg/day sitagliptin) for four days, with plasma DPP-4 activity determined 1 h and 24 h after dosing. In comparison with sham-operated rats, SNx rats exhibited reduced plasma DPP-4 activity (Figure 1). One hour after dosing, plasma DPP-4 activity was reduced by 75-90% with either linagliptin or sitagliptin in either sham-operated or SNx rats, with no difference observed between the two inhibitors (Figure 1).

Effect of DPP-4 inhibition on metabolic parameters in sham and SNx rats

Having confirmed dose-equivalency of the two DPP-4 inhibitors in SNx rats, sham and SNx rats were next randomized to receive either linagliptin 1.5 mg/kg/day, sitagliptin 20 mg/kg/day or vehicle commencing 10 days after subtotal nephrectomy or sham surgery and continuing for a further seven weeks. The functional characteristics of sham and SNx rats treated with vehicle, linagliptin and sitagliptin are shown in Table 1. Heart weights and kidney weights were increased in SNx rats relative to sham. Although fasting blood glucose was marginally reduced with linagliptin, it was unaffected by sitagliptin and HbA1c was unchanged in all treatment groups (Table 1). Plasma active GLP-1 levels were similarly increased by linagliptin and sitagliptin in both sham and SNx rats (Table 1).

Cardiac dysfunction in subtotally nephrectomized rats

Cardiac functional and structural parameters in sham-operated and SNx rats are shown in Table 2 and Figures 2 and 3. The indices of cardiac dysfunction that differed significantly between vehicle-treated SNx rats and vehicle-treated sham animals are summarized in Supplementary Table 1. This analysis revealed that SNx rats exhibited evidence of cardiac hypertrophy (increased heart weight:body weight ratio, anterior and posterior wall thickness, left ventricular mass and myocardial cross sectional area and decreased α-MHC:β-MHC ratio), cardiac fibrosis (increased collagen I mRNA and protein), diastolic dysfunction (elevated end diastolic pressure volume relationship [EDPVR]) and increased cardiac expression of both ANP and BNP (Supplementary Table 1).

DPP-4 inhibition attenuates cardiac dysfunction in subtotally-nephrectomized rats

In comparison with vehicle-treated SNx rats, EDPVR was significantly lower in SNx rats treated with either linagliptin or sitagliptin (Figure 2). This preservation of diastolic function was accompanied by an attenuation in the overexpression of type I fibrillar collagen at both the mRNA (Figure 3A) and protein (Figure 3B-H) levels. In contrast, neither cardiomyocyte size determined in H&E stained sections (Figure 3I-O) nor α-MHC:β-MHC ratio were changed with DPP-4 inhibition in SNx rats (α-MHC:β-MHC ratio [AU], vehicle 0.5±0.1 [p<0.05 vs. sham], linagliptin 0.7±0.1 [p=0.16 vs. vehicle] and sitagliptin 0.8±0.3 [p=0.33 vs. vehicle]).

Improved cardiac function with DPP-4 inhibition occurs independently of renal change in SNx rats

Analysis of a range of indicators of renal function and structure revealed that the beneficial effects of DPP-4 inhibition on cardiac diastolic dysfunction and fibrosis occurred independently of any change in the kidney. Table 3 shows the aortic systolic...
### TABLE 1. Metabolic characteristics of sham and subtotally-nephrectomized (SNx) rats treated with vehicle, linagliptin or sitagliptin.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body weight (g)</th>
<th>Left kidney weight (g)</th>
<th>Left kidney weight:body weight (%)</th>
<th>Heart weight (g)</th>
<th>Heart weight:body weight (%)</th>
<th>Lung weight (g)</th>
<th>24 h water intake (ml)</th>
<th>24 h urine output (ml)</th>
<th>HbA1c (%)</th>
<th>Fasting blood glucose (mmol/L)</th>
<th>Active GLP-1 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + vehicle</td>
<td>12</td>
<td>177±3</td>
<td>0.54±0.01</td>
<td>0.307±0.007</td>
<td>0.56±0.01</td>
<td>0.314±0.004</td>
<td>0.82±0.05</td>
<td>14±2</td>
<td>15±1</td>
<td>4.5±0.1</td>
<td>6.9±0.4</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>Sham + lina- gliptin</td>
<td>11</td>
<td>176±2</td>
<td>0.53±0.01</td>
<td>0.305±0.005</td>
<td>0.53±0.01</td>
<td>0.304±0.005</td>
<td>0.82±0.03</td>
<td>17±7</td>
<td>15±1</td>
<td>4.6±0.1</td>
<td>5.8±0.2</td>
<td>0.33±0.06</td>
</tr>
<tr>
<td>Sham + sitagliptin</td>
<td>14</td>
<td>179±3</td>
<td>0.54±0.01</td>
<td>0.304±0.004</td>
<td>0.56±0.01</td>
<td>0.315±0.004</td>
<td>0.80±0.02</td>
<td>12±2</td>
<td>14±1</td>
<td>4.6±0.1</td>
<td>6.1±0.1</td>
<td>0.28±0.05</td>
</tr>
<tr>
<td>SNx + vehicle</td>
<td>11</td>
<td>181±4</td>
<td>0.69±0.01</td>
<td>0.386±0.008</td>
<td>0.74±0.03</td>
<td>0.413±0.021</td>
<td>0.89±0.02</td>
<td>24±4</td>
<td>23±2</td>
<td>4.4±0.1</td>
<td>6.2±0.2</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>SNx + lina- gliptin</td>
<td>9</td>
<td>170±4</td>
<td>0.69±0.02</td>
<td>0.406±0.014</td>
<td>0.73±0.04</td>
<td>0.431±0.017</td>
<td>0.86±0.02</td>
<td>27±4</td>
<td>25±2</td>
<td>4.5±0.1</td>
<td>5.6±0.3</td>
<td>0.43±0.06</td>
</tr>
<tr>
<td>SNx + sitagliptin</td>
<td>8</td>
<td>167±6</td>
<td>0.61±0.04</td>
<td>0.363±0.024</td>
<td>0.71±0.05</td>
<td>0.437±0.040</td>
<td>0.85±0.02</td>
<td>17±3</td>
<td>19±1</td>
<td>4.3±0.1</td>
<td>6.0±0.3</td>
<td>0.53±0.15</td>
</tr>
</tbody>
</table>

*p<0.001 vs. sham + vehicle, †p<0.001 vs. sham + linagliptin, ‡p<0.001 vs. sham + sitagliptin, §p<0.01 vs. SNx + vehicle, ¶p<0.01 vs. SNx + linagliptin, ‖p<0.01 vs. sham + linagliptin, ƒp<0.01 vs. sham + sitagliptin,  ‍p<0.05 vs. SNx + linagliptin, ′p<0.05 vs. sham + vehicle, ″p<0.05 vs. sham + linagliptin, ′′p<0.01 vs. SNx + vehicle.

### TABLE 2. Cardiac function in sham and subtotally nephrectomized (SNx) rats treated with vehicle, linagliptin or sitagliptin.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Heart rate (bpm)</th>
<th>ESP (mmHg)</th>
<th>EDP (mmHg)</th>
<th>Tau Logistic (msec)</th>
<th>dp/dt + (mmHg/sec)</th>
<th>dp/dt - (mmHg/sec)</th>
<th>LVDD (cm)</th>
<th>LVDS (cm)</th>
<th>FS (%)</th>
<th>PWT (cm)</th>
<th>AW'T (cm)</th>
<th>LV mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + vehicle</td>
<td>12</td>
<td>327±6</td>
<td>130±4</td>
<td>9±0.7</td>
<td>9±0.2</td>
<td>7487±317</td>
<td>7341±393</td>
<td>0.56±0.02</td>
<td>0.23±0.01</td>
<td>58±2</td>
<td>0.155±0.008</td>
<td>0.127±0.006</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>Sham + lina- gliptin</td>
<td>11</td>
<td>299±12</td>
<td>126±4</td>
<td>9±0.6</td>
<td>9±0.5</td>
<td>6912±315</td>
<td>7593±303</td>
<td>0.58±0.01</td>
<td>0.25±0.01</td>
<td>56±2</td>
<td>0.162±0.006</td>
<td>0.126±0.005</td>
<td>0.48±0.03</td>
</tr>
<tr>
<td>Sham + sitagliptin</td>
<td>14</td>
<td>323±12</td>
<td>127±4</td>
<td>9±0.6</td>
<td>9±0.3</td>
<td>7389±447</td>
<td>8325±647</td>
<td>0.55±0.01</td>
<td>0.23±0.01</td>
<td>58±2</td>
<td>0.157±0.009</td>
<td>0.123±0.005</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>SNx + vehicle</td>
<td>11</td>
<td>304±15</td>
<td>163±9</td>
<td>11±0.6</td>
<td>10±0.6</td>
<td>8520±511</td>
<td>1144±1162</td>
<td>0.58±0.01</td>
<td>0.22±0.02</td>
<td>62±3</td>
<td>0.181±0.007</td>
<td>0.168±0.007j</td>
<td>0.64±0.04j</td>
</tr>
<tr>
<td>SNx + lina- gliptin</td>
<td>9</td>
<td>289±7</td>
<td>179±6</td>
<td>11±1.1</td>
<td>11±0.6</td>
<td>7946±352</td>
<td>9344±814</td>
<td>0.56±0.02</td>
<td>0.20±0.01</td>
<td>64±2</td>
<td>0.193±0.010</td>
<td>0.146±0.010</td>
<td>0.57±0.04ik</td>
</tr>
<tr>
<td>SNx + sitagliptin</td>
<td>8</td>
<td>295±6</td>
<td>151±13</td>
<td>10±0.7</td>
<td>11±0.6</td>
<td>7723±707</td>
<td>8417±1145</td>
<td>0.55±0.03</td>
<td>0.23±0.02</td>
<td>58±3</td>
<td>0.180±0.017</td>
<td>0.152±0.0088</td>
<td>0.55±0.05</td>
</tr>
</tbody>
</table>

ESP = end systolic pressure, EDP = end diastolic pressure, LVDD = left ventricular internal diameter in diastole, LVDS = left ventricular internal diameter in systole, FS = fractional shortening, PWT = posterior wall thickness, AW'T = anterior wall thickness.

*p<0.001 vs. sham + vehicle, †p<0.001 vs. sham + linagliptin, ‡p<0.001 vs. sham + sitagliptin, §p<0.0001 vs. sham + vehicle, ¶p<0.0001 vs. sham + linagliptin, ‖p<0.0001 vs. sham + sitagliptin, ƒp<0.0001 vs. sham + vehicle,  ‍p<0.0001 vs. sham + linagliptin, ′p<0.01 vs. sham + vehicle, ″p<0.05 vs. sham + linagliptin, ′′p<0.01 vs. sham + vehicle, ′′′p<0.05 vs. sham + linagliptin, ′′′′p<0.01 vs. sham + vehicle, ′′′′′p<0.05 vs. sham + linagliptin.
and diastolic pressures, GFR and urine protein in sham-operated and SNx rats. While, as expected, blood pressure and proteinuria were increased and GFR was reduced in SNx rats in comparison with their sham-operated counterparts, neither linagliptin nor sitagliptin significantly affected any of these parameters (Table 3). Histologically, surgical renal mass ablation in SNx rats resulted in an increase in deposition of extracellular matrix within the glomeruli (Figure 4A-G) and cortical tubulointerstitium (Figure 4H-N) and a decrease in glomerular capillary density (Figure 4O-U). None of these markers of renal structural injury were affected by either linagliptin or sitagliptin in either sham or SNx rats (Figure 4).

Neither linagliptin nor sitagliptin increase urinary excretion of renal toxicity markers

To further examine the “safety” of these agents when used in the context of renal impairment, multi-analyte profiling of urine samples was performed using a well-established panel of 12 renal toxicity biomarkers [27, 28]. Of the screen of 12 markers, seven were increased in SNx rats in comparison with sham (ß2-microglobulin, calbindin, clusterin, cystatin C, kidney injury molecule-1 [KIM-1], osteopontin and tissue inhibitor of metalloproteinases-1 [TIMP-1]), one was reduced (epi-}

### TABLE 3. Blood pressure and renal function in sham and subtotally-nephrectomized (SNx) rats treated with vehicle, linagliptin or sitagliptin.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Aortic systolic pressure (mmHg)</th>
<th>Aortic diastolic pressure (mmHg)</th>
<th>GFR (ml/min/kg)</th>
<th>Proteinuria (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + vehicle</td>
<td>12</td>
<td>122±6</td>
<td>96±6</td>
<td>4.9±0.4</td>
<td>1.7 (0.9-2.9)</td>
</tr>
<tr>
<td>Sham + linagliptin</td>
<td>11</td>
<td>126±5</td>
<td>98±3</td>
<td>5.3±0.4</td>
<td>1.8 (1.3-2.0)</td>
</tr>
<tr>
<td>Sham + sitagliptin</td>
<td>14</td>
<td>132±4</td>
<td>103±4</td>
<td>4.8±0.5</td>
<td>1.9 (0.5-2.5)</td>
</tr>
<tr>
<td>SNx + vehicle</td>
<td>11</td>
<td>174±10&lt;abc&gt;</td>
<td>128±6&lt;cd&gt;</td>
<td>2.7±0.4&lt;abdf&gt;</td>
<td>40.9 (2.9-113.4)&lt;abf&gt;</td>
</tr>
<tr>
<td>SNx + linagliptin</td>
<td>9</td>
<td>182±13&lt;abc&gt;</td>
<td>134±9&lt;cd&gt;</td>
<td>2.2±0.2&lt;abf&gt;</td>
<td>52.7 (5.5-189.2)&lt;abf&gt;</td>
</tr>
<tr>
<td>SNx + sitagliptin</td>
<td>8</td>
<td>172±17&lt;cd&gt;</td>
<td>128±9&lt;cd&gt;</td>
<td>2.3±0.5&lt;abf&gt;</td>
<td>37.3 (1.6-178.3)&lt;abf&gt;</td>
</tr>
</tbody>
</table>

GFR = glomerular filtration rate, skew distributed proteinuria data shown as median (range).

*p<0.001 vs. sham + vehicle, †p<0.001 vs. sham + linagliptin, ‡p<0.01 vs. sham + sitagliptin, §p<0.01 vs. sham + vehicle, ¶p<0.01 vs. sham + linagliptin, ♂p<0.001 vs. sham + sitagliptin.
dermal growth factor [EGF]), two were unaffected (glutathione-S-transferase µ [GST-µ] and vascular endothelial growth factor-A [VEGF-A]) and two were below the level of detection (GST-α and neutrophil gelatinase-associated lipocalin [NGAL]) (Figure 5). As with the conventional markers of renal injury, none of these sensitive parameters were altered with either linagliptin or “renally adjusted” sitagliptin (Figure 5).

Discussion

In recent years, inhibitors of the enzymatic activity of DPP-4 have become widely employed in the clinic for their blood glucose lowering effects in individuals with Type 2 diabetes. Because of the broad tissue distribution of the GLP-1R and because of the potential for DPP-4 inhibitors to augment the activity of alternative substrates, it has been postulated that this class of agents may exert biological effects that extend beyond the regulation of glucose homeostasis [29]. Against this background, two agents, differentiated by their method of elimination, were employed to investigate the glucose-independent effects of DPP-4 inhibition in a well-established experimental model of CKD and HFpEF. The consistent improvement in cardiac function in the context of apparent “renal neutrality” of either DPP-4 inhibitor, at the doses administered in our rodent model, attests to the organ-specific anti-fibrotic effects of this therapeutic strategy [6, 7, 13] and suggests that the clinical utility of incretin-based therapies may extend beyond the diabetic population.

HFpEF is common in the dialysis population where it can account for more cases of heart failure than systolic dysfunction [30]. Unfortunately, whereas ACE inhibitors and angiotensin II receptor blockers represent established evidence-based therapies for the treatment of either systolic heart failure or CKD, they provide little if any benefit in the treatment of
FIGURE 3. Effects of DPP-4 inhibition on cardiac structure in sham and subtotaly-nephrectomized (SNx) rats (n=8-14/group). (A) Cardiac collagen I mRNA. (B-G) Representative heart sections immunostained for collagen I from (B) sham + vehicle, (C) sham + linagliptin, (D) sham + sitagliptin, (E) SNx + vehicle, (F) SNx + linagliptin, (G) SNx + sitagliptin. Original magnification x400. (H) Quantitation of cardiac collagen I protein. (I-N) Representative H&E-stained heart sections from (I) sham + vehicle, (J) sham + linagliptin, (K) sham + sitagliptin, (L) SNx + vehicle, (M) SNx + linagliptin, (N) SNx + sitagliptin. Original magnification x400. (O) Myocyte area. AU=arbitrary units.

*p<0.01 vs. sham + vehicle, sham + linagliptin or sham + sitagliptin; †p<0.01 vs. SNx + vehicle; ‡p<0.001 vs. SNx + vehicle; §p<0.05 vs. sham + vehicle, sham + linagliptin or sham + sitagliptin; ¶p<0.05 vs. SNx + vehicle, || p<0.05 vs. sham + vehicle.
FIGURE 4. Renal structure in sham and subtotally-nephrectomized (SNx) rats treated with vehicle, linagliptin or sitagliptin (n=8-14/group).

(A-F) Representative PAS-stained kidney sections from (A) sham + vehicle, (B) sham + linagliptin, (C) sham + sitagliptin, (D) SNx + vehicle, (E) SNx + linagliptin, (F) SNx + sitagliptin. Original magnification x400. (G) Glomerulosclerosis index. (H-M) Representative kidney sections immunostained for collagen IV from (H) sham + vehicle, (I) sham + linagliptin, (J) sham + sitagliptin, (K) SNx + vehicle, (L) SNx + linagliptin, (M) SNx + sitagliptin. Original magnification x160. (N) Quantitation of cortical tubulointerstitial collagen IV. (O-T) Representative kidney sections immunostained with the endothelial-specific antibody JG-12 from (O) sham + vehicle, (P) sham + linagliptin, (Q) sham + sitagliptin, (R) SNx + vehicle, (S) SNx + linagliptin, (T) SNx + sitagliptin. Original magnification x400. (U) Quantitation of glomerular JG-12 immunostaining. AU=arbitrary units. *p<0.0001 vs. sham + vehicle, sham + linagliptin or sham + sitagliptin; †p<0.05 vs. sham + vehicle, sham + linagliptin or sham + sitagliptin.
HFpEF [31]. Given the need for new treatments, the effects of DPP-4 inhibition on cardiac and renal structure and function were investigated in subtotally-nephrectomized rats, an experimental model with a pedigree in therapeutics development [32]. In both human CKD and in subtotally-nephrectomized rats, pressure overload, uremia, salt retention and sympathetic nervous system activation act in concert to induce changes within the myocardial architecture characterized by deposition of fibrillar collagens, hypertrophy of the cardiac myocytes and obliteration of the microvessels [8, 33-35]. These structural changes are manifested as decreased chamber compliance, most readily appreciated as impairment in the passive phase of cardiac relaxation and reflected in an increase in the slope of the left ventricular EDPVR. In the present study, DPP-4 inhibition improved cardiac chamber relaxation and reduced cardiac fibrosis without altering myocyte hypertrophy, analogous to anti-fibrotic actions previously described in the liver [36], heart [37] and even the kidney [13].

The presence of hypertrophic changes without significant alteration in cardiac chamber dimensions suggests that pressure rather than volume overload is the primary mechanism underlying cardiac dysfunction in the subtotally-nephrectomized rat, an observation consistent with earlier findings [38]. Although volume overload is well-recognized in patients with ESRD, it has also been reported to occur in approximately half of patients with stage 3-5 CKD, where it correlates inversely with GFR decline [39]. In the present study, the absence of echocardiographic indices of volume overload in SNx rats may relate to the relatively preserved GFR or the short duration of renal dysfunction.

Although a number of studies have described a cardioprotective effect of DPP-4 inhibition in pre-clinical models (reviewed in [29]) the mechanisms underlying the cardiac effects of DPP-4 inhibition in heart failure remain incompletely resolved. For instance, in a previous report investigators attributed the beneficial actions of DPP-4 inhibition in HFpEF to both local effects on the activity of the alternative DPP-4 substrate, stromal cell-derived factor-1 (SDF-1α) and systemic effects of enhanced GLP-1 activity [6]. In a separate study, investigators reported that ligand-engagement of the GLP-1R results in ANP-dependent vasorelaxation [40].

The present study adds to this body of literature by demonstrating an attenuation of cardiac fibrosis by two separate DPP-4 inhibitors in an established model of CKD.

Since myocardial fibrosis, left ventricular hypertrophy and systemic hypertension commonly coexist in patients with CKD [41], the interrelationship between these processes may not easily be unraveled. Interestingly, however, a previous study of SNx rats noted a correlation between systemic hyper-

FIGURE 5. Urinary excretion of kidney injury markers in sham and subtotally nephrectomized (SNx) rats treated with vehicle, linagliptin or sitagliptin (n=8-14/group). *p<0.05 vs. sham + vehicle, sham + linagliptin or sham + sitagliptin; †p<0.01 vs. sham + vehicle, sham + linagliptin or sham + sitagliptin; ‡p<0.001 vs. sham + vehicle, sham + linagliptin or sham + sitagliptin.
tension and left ventricular mass but not with cardiac fibrosis [38], which is analogous to the present findings where cardiac collagen I accumulation was attenuated with DPP-4 inhibition without significant change in blood pressure or myocyte hypertrophy. Similarly, an earlier study of a murine model of Type 2 diabetes also reported that the DPP-4 inhibitor, sitagliptin attenuated indices of myocardial fibrosis without affecting cardiomyocyte size [42]. By way of contrast, ACE inhibition may improve cardiac hypertrophy without affecting cardiac interstitial fibrosis in SNx rats [43]. The combination of renin angiotensin system (RAS)-blockade and DPP-4 inhibition has been reported to attenuate albuminuria in a mouse model of diabetic nephropathy [44]. Whether the combination may similarly exert additive or synergistic effects on cardiac function in CKD remains to be determined. In this regard, it is noteworthy that several studies have described a relationship between the DPP-4 substrate, GLP-1 and the RAS [45-47].

The cardiac dysfunction in the SNx rats in the present study may not have fully developed, recognizing that this was accompanied by impairment in the passive phase of diastole (EDPVR), whereas neither active relaxation (Tau) nor EDP were significantly altered. Although an unchanged EDP has been previously described in both humans [48] and animals with HFpEF [21], confirmation of the cardioprotective benefits of DPP-4 inhibition in more advanced models of CKD and of longer duration is required. This is especially important in light of the unexpected increase in hospitalization for heart failure with the DPP-4 inhibitor, saxagliptin, reported in the recent SAVOR-TIMI 53 trial [49]. Accordingly, although it is notable that only 2% of the participants in SAVOR-TIMI 53 had a GFR <30 ml/min [49], caution should be taken in the extrapolation of pre-clinical observations such as these to the clinic whilst the results of other outcome trials are awaited.

The lack of observed effect of either linagliptin or sitagliptin on either GFR decline or various histological parameters of renal fibrosis is consistent with earlier reports in rodents with renal insufficiency [13] and short-term studies in patients [10]. In contrast, however, other studies have described a renoprotective benefit of incretin-based therapies in rodent models of Type 2 diabetes [50] and also in insulinopenic models of diabetes [44, 51]. Moreover, a recent meta-analysis suggested that linagliptin was associated with a reduction in albuminuria in patients with Type 2 diabetes [52]. These superficially discordant observations may be explained, at least in part, by differences in DPP-4 inhibitor dose or in duration of therapy [53, 54]. Although the doses of DPP-4 inhibitors used in the present study were pragmatically selected based on earlier short-term studies in SNx rats, they were higher than the theoretical area under the curve equivalent in humans [13]. Thus, the effects of either linagliptin or sitagliptin on cardiac function, when administered at approved doses in patients, remain to be demonstrated. A further limitation to the present study is the use of female rats. Even though the experimental groups were studied contemporaneously, an estrogen effect on the observed phenotype cannot be excluded [55]. Nonetheless, a major strength of the present study is the establishment of a therapeutic disconnect between the renal and cardiac effects of DPP-4 inhibition, which may have particular relevance to the patient population with severe renal impairment or ESRD.

In summary, in the present study DPP-4 inhibition attenuated cardiac diastolic dysfunction in a rodent model of CKD. Recapitulation of these findings in alternative models of renal insufficiency and cardiac fibrosis may extend the applicability of this class of agents beyond the diabetic population.

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Conflicts of Interest

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