Cardiac DPP-4 inhibition by saxagliptin ameliorates isoproterenol-induced myocardial remodeling and cardiac diastolic dysfunction in rats

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1. Introduction

Cardiovascular disease is a major cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM) (1). Although diabetic microvascular complications have been shown to be reduced by strict glycemic control, it is not clear whether strict glycemic control can reduce macrovascular complications in diabetes patients (2–5).

Myocardial remodeling, including cardiac hypertrophy and fibrosis, plays an important role in cardiovascular disease, and is an independent risk factor for cardiovascular events (6,7). Diabetes mellitus is associated with increased myocardial remodeling, and classical or new generation antidiabetic agents have been reported to ameliorate myocardial remodeling via both glucose-dependent and glucose-independent actions in various animal models of heart failure (8).

Dipeptidyl peptidase-4 (DPP-4) inhibitors improve glucose metabolism by preventing the degradation of incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). While these agents have been used in the treatment of T2DM, a meta-analysis of randomized clinical trials showed that DPP-4 inhibition reduced the risk of cardiovascular events and all-cause mortality in patients with T2DM (9). Furthermore, recent animal studies demonstrated that DPP-4 inhibition ameliorated cardiac dysfunction and myocardial remodeling in non-diabetic animal models of cardiovascular injury (10,11).

Saxagliptin, a potent and selective DPP-4 inhibitor, is linked to DPP-4 by a covalent bond and is characterized by its slow dissociation from DPP-4 and long half-life (12). Saxagliptin is therefore expected to have a potent tissue membrane-bound DPP-4 inhibitory effect in various tissues. In the present study, we examined the effects of saxagliptin in situ cardiac DPP-4 activity. We also examined the effects of saxagliptin on isoproterenol-induced changes in the early stage such as, myocardial remodeling and cardiac diastolic dysfunction. Male SD rats treated with isoproterenol (1 mg/kg/day via osmotic pump) received vehicle or saxagliptin (17.5 mg/kg via drinking water) for 2 weeks. In situ cardiac DPP-4 activity was measured by a colorimetric assay. Cardiac gene expressions were examined and an echocardiographic analysis was performed. Saxagliptin treatment significantly inhibited in situ cardiac DPP-4 activity and suppressed isoproterenol-induced myocardial remodeling and the expression of related genes without altering the blood glucose levels. Saxagliptin also significantly ameliorated cardiac diastolic dysfunction in isoproterenol-treated rats. In conclusion, the inhibition of DPP-4 activity in cardiac tissue by saxagliptin was associated with suppression of myocardial remodeling and cardiac diastolic dysfunction independently of its glucose-lowering action in isoproterenol-treated rats. Cardiac DPP-4 activity may contribute to myocardial remodeling in the development of heart failure.

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inhibitory effect in various tissues. We previously demonstrated that saxagliptin exerts renoprotective effects through its potent inhibitory effects on renal DPP-4 activities in Dahl salt-sensitive hypertensive rats (13,14). In addition, a recent animal study demonstrated that saxagliptin treatment improved cardiac dysfunction in an experimental model of post-myocardial infarction without affecting the blood glucose level (15). In the present study, we examined the effects of treatment with saxagliptin on in situ cardiac DPP-4 activity and myocardial remodeling in a rat model of isoproterenol-induced heart failure. In addition, we also examined the effects of saxagliptin on isoproterenol-induced cardiac diastolic dysfunction in rats. We obtained evidence that saxagliptin inhibited cardiac DPP-4 activity and was associated with an improvement in myocardial remodeling and diastolic dysfunction without glycemic action in isoproterenol-treated rats.

2. Materials and methods

2.1. Animals

Male eight-week-old Sprague–Dawley (SD) rats (Charles River Japan, Inc., Yokohama, Japan) were used. Prior to the experiments, the rats were kept at 19–25 °C in 30–70% humidity under a 13-h light–dark cycle with ad libitum access to tap water and commercial chow (FR-2; Funabashi Farm, Chiba, Japan). All of the animal experiments were approved by the Committee for Animal Experiments of Kyowa Hakko Kirin Co., Ltd. All of the animals received humane care that complied with the Japanese Pharmacological Society’s “Guiding Principles for the Care and Use of Laboratory Animals”.

2.2. Saxagliptin monohydrate

Saxagliptin monohydrate (saxagliptin) was obtained from Bristol Myers Squibb (Pennington, NJ, USA). Saxagliptin was dissolved in distilled water (0.025 w/w) and was administered to rats with drinking water. The saxagliptin dose that was calculated from their daily water consumption was approximately 17.5 mg/kg/day. The dose was determined based on the findings of preliminary studies indicating the inhibitory effect of 17.5 mg/kg of saxagliptin on the plasma DPP-4 activity of rats.

2.3. Experimental procedures

Forty-five rats were divided into the following three groups: (1) normal group (vehicle); (2) control group (vehicle); and (3) saxagliptin (approximately 17.5 mg/kg/day). Isoproterenol (1 mg/kg/day) was administered for 2-weeks to all of the rats (except those in the normal group) by a subcutaneously implanted osmotic mini-pump (Alzet, model 202; Direx, Cupertino, CA). Saxagliptin treatment (via drinking water) was initiated at the same time. After the 2-week treatment, the rats were anesthetized with 3% isoflurane, blood samples were collected and their hearts were removed. The weight of each heart was measured. The blood and heart samples were used for the measurement of DPP-4 activity.

2.4. Echocardiography

At 2 weeks after the start of saxagliptin treatment, echocardiography was performed in rats anesthetized with 3% isoflurane using a 15 MHz ultrasound probe (MS200, VisualSonics, Toronto, Canada) and an ultrasound imaging system (Veo2100, VisualSonics, Toronto, Canada). M-mode recordings were performed through the left ventricular anterior and posterior wall at the level of the papillary muscles to measure the left ventricular end-diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), fractional shortening (FS), left ventricular ejection fraction, left ventricular posterior wall thickness (PWT) and the interventricular septum wall thickness (IVST). FS was calculated according to the following formula:

FS = \((LVDd - LVDs)/LVDd\) \times 100

The LV mass was calculated according to the following formula:

LV mass = 1.04 \times [(LVDd + PWT + IVST) \times (LVDd)]

Pulse wave Doppler-mode recordings were performed to measure the peak early left ventricular filling wave (MVE), the peak late or atrial filling wave velocity (MVA) and the E-wave deceleration time (DT).

2.5. The measurement of the plasma DPP-4 activity

The plasma samples were separated via centrifugation (1800 \times g for 20 min at 4 °C). The rats’ plasma DPP-4 activity levels were measured using a fluorometric assay with Gly-Pro-7-AMIDO-4-METHYLCOUMARIN (Gly-Pro-AMC) (PEPTIDE INSTITUTE, Osaka, Japan) substrate. A 25 μL volume of plasma diluted two-fold with distilled water was mixed with 25 μL of assay buffer (25 mmol/L of HEPES, 140 mmol/L of NaCl, 80 mmol/L of MgCl2/6H2O, 1 w/v% BSA, pH 7.8). To exclude the non-specific amino peptidase activity, plasma samples that were diluted two-fold with DPP-4 inhibitor-containing solution (10 μmol/L saxagliptin) were also used. The enzyme reaction was initiated by adding 50 μL of substrate solution (final concentration: 50 μmol/L Gly-Pro-AMC), followed by incubation for 20 min at room temperature. The reaction was then terminated by adding 50 μL of 10% acetic acid solution. The fluorescence intensity was measured using a Spectra Max M2e microplate reader (Molecular Devices, Sunnyvale, USA) at an excitation wavelength of 460 nm and an emission wave length of 390 nm. The DPP-4 activity was expressed as the amount of AMC generated after 20 min of incubation.

2.6. In situ cardiac DPP-4 activity

The in situ cardiac DPP-4 activity was measured in 5 rats of each group. The left ventricular chambers were frozen and embedded in OCT compound. The sections were cut using a cryostat and an ultrasound imaging system (Veo2100, VisualSonics, Toronto, Canada) and an ultrasound imaging system (Veo2100, VisualSonics, Toronto, Canada). M-mode recordings were performed through the left ventricular anterior and posterior wall at the level of the papillary muscles to measure the left ventricular end-diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), fractional shortening (FS), left ventricular ejection fraction, left ventricular posterior wall thickness (PWT) and the interventricular septum wall thickness (IVST). FS was calculated according to the following formula:

FS = \((LVDd - LVDs)/LVDd\) \times 100

The LV mass was calculated according to the following formula:

LV mass = 1.04 \times [(LVDd + PWT + IVST) \times (LVDd)]

Pulse wave Doppler-mode recordings were performed to measure the peak early left ventricular filling wave (MVE), the peak late or atrial filling wave velocity (MVA) and the E-wave deceleration time (DT).

2.7. Histological examination

The left ventricular chambers were fixed in 10 vol% neutral buffered formalin solution and embedded in paraffin. The paraffin-embedded sections were then stained with Masson’s trichrome to quantify the extent of the myocardial and perivascular fibrosis. The myocardial or perivascular fibrotic area was measured using the Aperio ImageScope and Color Deconvolution version 9 software programs. The ratio of myocardial or perivascular fibrosis in a randomized area was calculated.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Control</th>
<th>Saxagliptin</th>
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</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>395.5 ± 3.9</td>
<td>415.4 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>398.6 ± 4.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.09 ± 0.02</td>
<td>1.46 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart to body weight ratio, mg/g</td>
<td>2.76 ± 0.04</td>
<td>3.52 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.34 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>145.0 ± 4.2</td>
<td>147.3 ± 4.1</td>
<td>142.3 ± 3.3</td>
</tr>
<tr>
<td>Plasma DPP-4 activity, pmol</td>
<td>855 ± 67</td>
<td>1130 ± 84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>113 ± 18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The data represent the mean ± S.E.M. a: P < 0.05, b: P < 0.01 vs. the control group. c: P < 0.01 in comparison to the normal group (Student’s t-test). Plasma DPP-4 activity was expressed as the amount of AMC (pmol) generated after 20 min of incubation.

3. Results

3.1. The effects on blood glucose and plasma DPP-4 activity

The blood glucose levels in the control and normal groups were similar, and saxagliptin did not affect the blood glucose levels in the isoproterenol-treated rats. Isoproterenol treatment significantly increased the plasma DPP-4 activity in comparison to the normal group (855 ± 67 vs. 1130 ± 84 pmol; P < 0.05). Saxagliptin inhibited the plasma DPP-4 activities of the isoproterenol-treated rats by 90% (113 ± 18 pmol; P < 0.01 vs. the control group) (Table 1).

3.2. In situ cardiac DPP-4 activity

The DPP-4 activity in cardiac tissue was assessed by a colorimetric assay. The endothelium of the venous part of the capillary bed displayed DPP-4 activity and was stained red. The H-scores of the staining intensity in the normal and control groups did not differ to a statistically significant extent (8.12 ± 2.12 vs. 5.16 ± 0.62). Saxagliptin significantly suppressed the cardiac DPP-4 activity of the isoproterenol-treated rats (Fig. 1) (2.12 ± 0.21; P < 0.05 vs. the control group).

3.3. The effects of saxagliptin on myocardial remodeling including cardiac hypertrophy and fibrosis, in isoproterenol-treated rats

The weight of the heart relative to the body weight indicated that isoproterenol treatment induced cardiac hypertrophy in rats. Saxagliptin significantly suppressed the increase in the relative weight of the heart in the isoproterenol-treated rats (Table 1). A histopathological examination showed that the areas of myocardial and perivascular fibrosis in the left ventricle were significantly increased in the control group in comparison to the normal group. Saxagliptin significantly attenuated the increase in the perivascular fibrotic area and tended to attenuate the area of myocardial fibrosis.

Fig. 1. The effects of saxagliptin on in situ cardiac DPP-4 activity in isoproterenol-treated rats. The data represent the mean ± S.E.M. (n = 15). *: P < 0.01 vs. the control group (Student’s t-test). Abbreviations: N.S., not significant.
after the 2-week administration of isoproterenol in comparison to trichrome. The data represent the mean (FS) and LV mass were significantly increased in the control group in comparison to the normal group in Student’s t-test. In the gene expression study, isoproterenol also significantly ameliorated the decrease in the E/A ratio in the isoproterenol-treated rats (Table 3).

4. Discussion

In the present study, saxagliptin inhibited both in situ cardiac DPP-4 activity staining and myocardial remodeling in a glucose-independent manner in a rat model of isoproterenol-induced heart failure. Furthermore, saxagliptin ameliorated isoproterenol-induced cardiac diastolic dysfunction in rats. From these findings, we hypothesize that the inhibition of DPP-4 activities in the cardiac tissue by saxagliptin may suppress the myocardial remodeling independently of its glucose-lowering action, and thereby ameliorate cardiac diastolic dysfunction in rats with isoproterenol-induced heart failure.

In the present study, we measured the cardiac DPP-4 activity using an in situ activity staining method and detected DPP-4 staining that was localized in the endothelium of the venous capillary vessels in cardiac tissues. Saxagliptin significantly inhibited cardiac DPP-4 activities in isoproterenol-treated rats. To our knowledge, this is the first study to demonstrate the suppressive effect of DPP-4 inhibitor on cardiac DPP-4 activity staining. Saxagliptin treatment improves eNOS coupling in the aortic and glomerular endothelium of animal models of hypertension (16). Recent clinical studies have demonstrated that endothelial dysfunction was closely associated with diastolic dysfunction in patients with type 2 diabetes, chronic kidney disease or heart failure.

3.4. The effects of saxagliptin on the echocardiographic parameters in isoproterenol-treated rats

The left ventricular end-systolic dimension (LVDs), interventricular septum wall thickness (IVST), fractional shortening (FS) and LV mass were significantly increased in the control group after the 2-week administration of isoproterenol in comparison to the normal group. There was no difference in the LVDs, IVST, FS or LV mass values of the control and saxagliptin-treated groups. The peak early left ventricular filling wave (MVE)/peak late or atrial filling wave velocity (E/A) ratio and E-wave deceleration time (DT) were significantly decreased in the control group in comparison to the normal group. Saxagliptin treatment significantly ameliorated the decrease in the E/A ratio in the isoproterenol-treated rats (Table 3).

### Table 2
The effects of saxagliptin on cardiac gene expression in isoproterenol-treated rats.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal</th>
<th>Control</th>
<th>Saxagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP, /r18s</td>
<td>0.75 ± 0.15</td>
<td>8.00 ± 1.24</td>
<td>4.47 ± 1.05</td>
</tr>
<tr>
<td>IL-6, /r18s</td>
<td>0.77 ± 0.05</td>
<td>3.63 ± 0.66</td>
<td>2.09 ± 0.41</td>
</tr>
<tr>
<td>IGF-1, /r18s</td>
<td>0.80 ± 0.07</td>
<td>1.56 ± 0.28</td>
<td>1.16 ± 0.16</td>
</tr>
<tr>
<td>Collagen I, /r18s</td>
<td>1.01 ± 0.05</td>
<td>2.81 ± 0.47</td>
<td>2.20 ± 0.47</td>
</tr>
<tr>
<td>Collagen III, /r18s</td>
<td>0.83 ± 0.05</td>
<td>2.26 ± 0.35</td>
<td>1.72 ± 0.32</td>
</tr>
</tbody>
</table>

The data represent the mean ± S.E.M. a: P < 0.01 in comparison to the normal group (Student’s t-test). b: P < 0.05 in comparison to the control group (Student’s t-test).

### Table 3
The effects of saxagliptin on the echocardiographic parameters of isoproterenol-treated rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Control</th>
<th>Saxagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>354.5 ± 5.6</td>
<td>424.6 ± 8.2</td>
<td>430.2 ± 10.9</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>8.06 ± 0.17</td>
<td>7.68 ± 0.16</td>
<td>7.21 ± 0.14</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>4.35 ± 0.14</td>
<td>2.24 ± 0.28</td>
<td>2.07 ± 0.16</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>2.47 ± 0.12</td>
<td>2.78 ± 0.09</td>
<td>2.86 ± 0.13</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>2.81 ± 0.08</td>
<td>4.50 ± 0.14</td>
<td>4.25 ± 0.13</td>
</tr>
<tr>
<td>EF, %</td>
<td>75.3 ± 1.5</td>
<td>94.0 ± 1.1^a</td>
<td>94.0 ± 1.4</td>
</tr>
<tr>
<td>FS, %</td>
<td>46.0 ± 1.5</td>
<td>71.2 ± 2.1^a</td>
<td>71.2 ± 2.4</td>
</tr>
<tr>
<td>E/A, ratio</td>
<td>1.92 ± 0.06</td>
<td>1.47 ± 0.06^a</td>
<td>1.68 ± 0.06^b</td>
</tr>
<tr>
<td>DT, ms</td>
<td>36.8 ± 0.9</td>
<td>28.8 ± 1.4^a</td>
<td>31.0 ± 1.4</td>
</tr>
</tbody>
</table>

LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; PWT, left ventricular posterior wall thickness; IVST, interventricular septum wall thickness; EF, left ventricular ejection fraction; FS, fractional shortening; E, peak early left ventricular filling wave; A, peak late or atrial filling wave velocity; DT, E-wave deceleration time. The data represent the mean ± S.E.M. a: P < 0.01 in comparison to the normal group (Student’s t-test). b: P < 0.05 in comparison to the control group (Student’s t-test).
failure (17–19). Saxagliptin might therefore exert a cardioprotective effect through a direct inhibitory action on cardiac endothelial DPP-4 activities in isoproterenol-treated rats.

In the present study, saxagliptin treatment prevented the cardiac hypertrophy and fibrosis induced by the continuous infusion of isoproterenol. This effect was accompanied by the suppressed expression of genes related to hypertrophy, such as ANP, IL-6 and IGF-1 and pro-fibrotic factors, such as collagen I and collagen III. Myocardial remodeling, including cardiac hypertrophy and fibrosis, has been demonstrated to significantly contribute to cardiac dysfunction in various types of heart disease (20,21). Cardiac hypertrophy is an important compensatory mechanism of the heart that occurs in response to pathological stresses such as hypertension, diabetes, myocardial injury or increased cardiac work. Persistent hypertrophy is associated with cardiac dysfunction. Previous studies have demonstrated that increased cardiac fibrosis can induce diastolic dysfunction (22,23). Thus, the improvement of cardiac hypertrophy and fibrosis by saxagliptin that was observed in this study might have been responsible for the improved left ventricular function. Thus, saxagliptin might suppress cardiac fibrosis through the suppression of cellular phenotypic modulation by the inhibition of endothelial DPP-4 activity in cardiac tissue, independently of its glycemic action.

Recent studies have demonstrated that DPP-4 activities in cardiac tissue were upregulated in experimental models of heart failure with both cardiac diastolic and systolic dysfunction (24,25). These findings suggest that an increase in cardiac DPP-4 activity may be associated with the development of heart failure. On the other hand, the present study showed similar levels of cardiac DPP-4 activity in the normal and control groups of our rat model of isoproterenol-induced heart failure. In the present study, the isoproterenol induced cardiac diastolic dysfunction but did not induced cardiac systolic dysfunction (the decrease of EF and FS). In rat model of isoproterenol-induced heart failure, histopathological examination showed that mild or moderate myocardial fibrosis of left ventricle 2 weeks after the isoproterenol treatment. These changes of early stage in the present study though to be milder than the degrees of injury in the heart failure models of previous studies. Thus, the activities of DPP-4 in cardiac tissue might depend on the severity of cardiac injury.

DPP-4 is known to cleave a wide range of substrates as well as incretin hormones, such as NPY, ANP/BNP, SDF-1 and HMGBl. These substrates have been reported to exert GLP-1-independent tissue protective effects (26). Shigeta et al. demonstrated that DPP-4 inhibition exerts a cardioprotective action via membrane-bound DPP-4/SDF-1a-dependent action in diabetic rats with diastolic left ventricular dysfunction (27). Furthermore, DPP-4 is widely expressed on subset of various cell types such as epithelial cells, macrophage and leukocyte as well as on the endothelial cells. In addition, DPP-4 is able to interact with proteins such as adenosine deaminase and mannose-6 phosphate/IGF-IIR and is reported to exhibit functions in the immune response and endothelial cell damage (28–30). Thus, saxagliptin might also exert cardioprotective effects (in part) via GLP-1-independent tissue-protective actions in rats with heart failure.

In a large scale prospective clinical study, the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR TIMI 53) study, saxagliptin did not increase or reduce the risk for major adverse cardiovascular event (MACE) in patients with type 2 diabetes, when added to the patients receiving current standard care (31). However, in SAVOR-TIMI 53 study, an unexpected increased risk of hospitalization for heart failure was found in the saxagliptin group. The explanation for this hospitalization for heart failure remains unknown. In the rat model of heart failure, saxagliptin did not worsen heart failure but exerted cardioprotective effects although the efficacies were restricted. The clinical effect of saxagliptin might become apparent by evaluating the effect on early stage of heart failure or by measuring biomarkers and echocardiographic studies in large outcome trials. In addition, further research needs to investigate the longer-term or reversing (therapeutic) effects of saxagliptin on heart failure.

In conclusion, the highly potent and tight-binding DPP-4 inhibitor saxagliptin inhibited both in situ cardiac DPP-4 activity staining and myocardial remodeling, including cardiac hypertrophy and fibrosis, in rats with isoproterenol-induced heart failure. Furthermore, saxagliptin ameliorated cardiac diastolic dysfunction in the isoproterenol-treated rats. Our findings therefore suggest that the cardiac DPP-4 inhibition induced by saxagliptin may ameliorate cardiac diastolic dysfunction via the suppression of myocardial remodeling in rats with isoproterenol-induced heart failure.

Conflicts of interest

The authors declare no conflicts of interest.

References


