The contributions of dipeptidyl peptidase IV to inflammation in heart failure

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New & Noteeworthy

This study shows that dipeptidyl peptidase IV (DPPIV) inhibition improves cardiac perfusion and inflammation in an experimental model of heart failure (HF). Furthermore, this study demonstrates that circulating DPPIV activity correlates with the inflammatory biomarker CCL2 in HF patients and that splenocytes might contribute to the increased levels of circulating DPPIV in HF.

Dipeptidyl Peptidase IV (DPPIV), also known as CD26, is a cell-surface type II serine protease that selectively cleaves NH₂-terminal dipeptides from peptides with a proline or alanine in amino acid position 2 (18). DPPIV, which is known as a T cell differentiation antigen, is expressed on the surface of several cell types, including epithelial and endothelial cells. Notably, a soluble form of the enzyme is also found in plasma. Through its catalytic action, DPPIV modulates the bioavailability of several regulatory peptides, neuropeptides, circulating hormones, and chemokines (24). The most well-studied substrates of DPPIV are the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), which play an essential role in insulin secretion and glycemic control (10). Therefore, a great deal of research has been dedicated to exploring the therapeutic potential of DPPIV inhibition for the treatment of type 2 diabetes, which led to the approval of the first DPPIV inhibitor, sitagliptin, by the FDA in 2006 (34).

Emerging evidence suggests that DPPIV inhibitors, also known as gliptins, may provide cardiovascular benefits beyond glycemic control (1, 3, 4, 13, 23, 40). In fact, genetic deletion or pharmacological inhibition of DPPIV improves cardiovascular outcomes after myocardial infarction in both normoglycemic and diabetic mice (42). In addition, increased DPPIV activity in the plasma significantly correlates with poorer prognosis, including cardiac dysfunction and mortality in patients with heart failure (HF) (13, 26, 46).

HF is a complex heterogeneous syndrome characterized by the activation of different neurohumoral, metabolic, and immune mechanisms (12, 21, 30). Inflammation has been suggested to play a key role in the pathogenesis, progression, severity, and prognosis of HF (14). A local inflammatory response is necessary for tissue healing after myocardial injury; however, an exaggerated and/or long-term response seems to be deleterious (14). During a chronic proinflammatory state, cardiac remodeling/dysfunction has been found to correlate with increased inflammatory markers such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-C chemokine ligand 2 (CCL2) (5, 15, 51).

Despite the aforementioned data, the contributions of DPPIV to inflammation in HF have not yet been elucidated. Therefore, this study tested the hypothesis that the cardioprotective effects of DPPIV inhibition after myocardial injury in rats are associated with reduced cardiac inflammation. Addi-
tionally, we examined whether circulating DPPIV activity correlates with the levels of systemic inflammatory markers in HF patients. Finally, we investigated whether leukocytes and/or splenocytes may be one of the sources of circulating DPPIV in HF.

**MATERIALS AND METHODS**

Animal protocols and surgical procedures. All experiments were performed in accordance with the ethical principles in animal research of the Brazilian College of Animal Experimentation and were approved by the Institutional Animal Care and Use Committee. Male Wistar rats (200–250 g) were subjected to myocardial injury by ablation of the left ventricle (LV) using a radiofrequency catheter as described previously (2, 13, 19). Briefly, rats were anesthetized with halothane, and the heart was exposed after a left thoracotomy at the fourth intercostal space. A catheter was placed on the anterolateral wall of the LV, and lesions were created using high-frequency currents (1,000 KHz, 12 W, over 10 s) generated by a conventional radiofrequency generator (model TEB RF10; Tecnologia Eletrônica Brasileira, São Paulo, Brazil). Sham-operated animals underwent the same procedure but were mock ablated. Sitagliptin (Merck, Kenilworth, NJ) was administrated by oral gavage (200 mg/kg 2 times a day) for 6 wk after cardiac injury as described previously (13). Both the sham-operated control and HF groups received vehicle treatment (water). Since one of the main goals of this study was to test the hypothesis that the cardioprotective effects of DPPIV inhibition after myocardial injury are accompanied by reduced cardiac inflammation, we have not examined the effects of sitagliptin on sham rats. The effects of DPPIV inhibition on cardiac function of control rats have been previously evaluated by Takahashi et al. (49). These authors have found that sham rats treated with the DPPIV inhibitor vildagliptin do not display significant improvements on cardiac function compared with sham treated with vehicle.

Glucose tolerance test. Six weeks after cardiac surgery, the rats were fasted for 8 h and placed in individual cages. Blood glucose was measured at baseline, 15, 30, 60, 90, and 120 min after an intraperitoneal administration of 2 g/kg of glucose. The blood glucose level was determined from their tail vein using the ACCU-CHECK Performa meter (Roche Diagnostics, Mannheim, Germany). The area under the curve (AUC) was determined for quantification of the glucose tolerance test (GTT).

Assessment of cardiac function. Six weeks after the surgical procedures, invasive hemodynamic studies were performed as described previously (19) with a pressure-volume catheter (model 1.4 French SPR 839; Millar Instruments, Houston, TX) that was positioned in the LV. In brief, anesthetized rats (50 mg/kg ketamine and 10 mg/kg ip xylazine) were placed on a heated rodent operating table (37°C), and a microtip pressure-volume catheter was positioned into the LV cavity by means of right carotid artery catheterization. The data were acquired for computer analysis (PVAN Software; Millar Instruments) using the LabChart 7 Software System (PowerLab; ADInstruments, Bella Vista, New South Wales, Australia).

Assessment of cardiac perfusion. Cardiac perfusion was evaluated by single-photon emission computed tomography (SPECT) imaging in a set of rats that were not subjected to invasive hemodynamic studies. Radiosynthesis was performed by eluting sodium [99mTc]pertechnetate (N99mTcO4) from a 99Mo/99mTc generator (IPEN-TEC). The eluate was then used for labeling methoxysobutylinonitrile (MIBI) (Cardiolite; DuPont-Merck Pharmaceutical, Billerica, MA). Na99mTcO4 (1.11–1.85 GBq in 2 ml) was added to the lyophilized kit and heated in boiling water for 10 min and then allowed to reach room temperature. The manufacturer’s quality control recommendations were followed, and the radiochemical was injected only when purity was higher than 95%. After radiosynthesis, the rats were anesthetized with 2–3% isoflurane in oxygen and injected intravenously (penile vein) with 20–35 MBq of 99mTc-MIBI. The rats were then allowed to wake up for a better radiopharmaceutical distribution. The rats were then once again anesthetized (30 min after 99mTc-MIBI injection) and positioned in the Triumum Tradiomodality scanner (Gamma Medica-Ideas, Northridge, CA) for small animal imaging. The rats were positioned based on planar X-ray topogram image with the heart in the center of the field of view of the SPECT. The images were acquired using 5 pinhole collimators with 64 projections of 30 s each and reconstructed using the OSEM algorithm with 5 interactions and 8 subsets. Image analysis was performed using PMOD software, and the segment score results of the heart perfusion were expressed as the relative percentage of the maximum uptake in the LV.

Preparation of heart homogenates. Hearts from rats were minced with razor blades and homogenized in a Polytron PT 2100 homogenizer (Kinematica) in an ice-cold buffer pH 7.4 containing 150 mM NaCl, 7.2 mM Na2HPO4, 2.8 mM NaH2PO4, 15 mM NaF, 50 mM Na4O7P2 × 10H2O, and Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, Rockford, IL). The homogenate was centrifuged at 300 g for 10 min at 4°C, and the supernatant was aliquoted and stored at –80°C. The protein concentration was determined using the Lowry method (27).

**SDS-PAGE and immunoblotting.** Protein samples were solubilized in Laemmli sample buffer, separated by SDS-PAGE, and then transferred to polyvinylidene fluoride (PVDF) membranes (Immobilon-P; Millipore, Bedford, MA). For immunoblotting, the membranes were incubated with 5% nonfat dry milk or 5% bovine serum albumin and

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<th>Table 1. The sequences of the primers used for SYBR Green real-time RT-PCR</th>
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<th>Table 2. The clinical characteristics of the heart failure population</th>
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0.1% Tween 20 in PBS, pH 7.4 for 1 h to block nonspecific antibody binding. Then, the blots were incubated overnight in primary antibodies diluted as described in 0.1% Tween 20 in PBS, pH 7.4. Antibodies against total and phospho-p38 (1:1,000) were purchased from Cell Signaling (Danvers, MA). The membranes were then washed five times with the blocking solution and incubated for 1 h with horseradish peroxidase-conjugated immunoglobulin secondary antibody (1: 2,000). Subsequently, the membranes were washed again and then rinsed in PBS. An enhanced chemiluminescence system (GE Healthcare, Marlborough, MA) was used to visualize the bands. The resolved bands were scanned using the ImageScanner (GE HealthCare) and quantified using ImageJ Software.

RNA isolation and real-time RT-PCR reaction. The gene expression of proinflammatory cytokines (TNF-α, IL-1β, and IL-6), the chemokine CCL2, and M1 [inducible nitric oxide synthase (iNOS); interferon regulatory factor 5 (Irf5)] and M2 (arginase-1) macrophage markers were examined by quantitative RT-PCR. Total RNA was isolated using Trizol Reagent (Thermo Fisher Scientific) according to the manufacturer’s instructions. First-strand cDNA synthesis was performed using Super-Script III Reverse Transcriptase following the manufacturer’s guidelines. The oligonucleotides were designed into different exons with a large intron between them to avoid amplifying genomic DNA. The quantitative RT-PCR reactions were performed using SYBR Green PCR Master Mix-PE (Thermo Fisher Scientific) on an ABI QuantStudio 12K flex machine (Thermo Fisher Scientific). All samples were assayed in duplicate. GAPDH was used as the control to normalize the results. The data were analyzed by the 2^–ΔΔCT method. The oligonucleotide primers that were used are described in Table 1.

Immunohistochemistry and immunofluorescence. The hearts were fixed with formaldehyde, paraffin embedded, and sectioned. An antigen retrieval step was used in all experiments, which included heating the samples in a citrate buffer (Spring-Bioscience) to 95°C for 30 min. After preincubation of heart sections with PBS solution containing 2% casein, the sections were incubated overnight with an anti-CD68 antibody (Abcam, Cambridge, UK) and then washed and incubated with secondary antibody. Immunostaining was visualized under a light microscope. The infiltration of CD68^+ cells was estimated by analyzing 15 images at ×200 magnification fields per animal near the lesion area. Positive staining was quantified and normalized by field area with ImageJ software. For dual fluorescence, primary anti-CD68 and anti-GLP1R (Santa Cruz Biotechnology, Dallas, TX) antibodies were used. Alexa Fluor 555 and Alexa Fluor 488 (Thermo Fisher Scientific) were used as the secondary antibodies. Immunofluorescence staining was detected using a Carl Zeiss 510 LMS confocal system connected to an Axiovert microscope.

Rat macrophage isolation and cell culture. Male Wistar rats (200–250 g) were injected intraperitoneally with 3 ml of 4% thioglycollate

Fig. 1. Evaluation of biometric parameters, cardiac function and dipeptidyl peptidase IV (DPPIV) activity in sham, heart failure (HF) rats, and HF rats treated with sitagliptin. A: the ratio between heart weight (mg) and body weight (BW; g) is shown. B: the percentage of lung water content as an index of pulmonary congestion is shown. The ejection fraction (C) and end-diastolic left ventricular (LV) pressure (D) are shown. Cardiac hemodynamic evaluations were performed as described in MATERIALS AND METHODS. DPPIV activity in the plasma (E) and the heart (F) was measured by colorimetry using a specific DPPIV substrate. Values are the means ± SE; n = 15 rats/group except for sham, in which there were 12 rats/group. *P < 0.05 vs. sham and #P < 0.05 vs. HF.
(TG) solution. TG is routinely used in studies with peritoneal exudate macrophages as an eliciting agent (11). After 96 h, the rats were anesthetized and their peritoneal cavity was washed twice with 20 ml of RPMI culture medium (Cultilab, Campinas, São Paulo, Brazil). After a gentle massage of the abdominal wall, the peritoneal fluid was collected and centrifuged at 300 g for 5 min at 4°C. A cell pellet was formed and suspended in 1 ml of lysis solution, pH 7.4 containing 150 mM NH₄Cl, 10 mM NaHCO₃, and 0.1 mM EDTA for 10 min at 4°C. Thereafter, the cells were centrifuged at 300 g for 30 s at 4°C and were suspended in 1 ml of RPMI medium twice for further use. In total, 1 × 10⁶ cells/ml were plated in a 24-well plate. After a 1-h incubation, the adherent cells were identified as macrophages. The macrophages were preincubated with 30 nM exendin-4 (Ex-4; Abcam) or vehicle for 30 min. Subsequently, 1 µg/ml of LPS (Escherichia coli 055:B5; Sigma) or vehicle was added to the cell culture medium. Three hours later, the culture medium was collected and ELISA kits were used to evaluate the levels of TNF-α, IL-1β, and IL-6 (R&D Systems; Minneapolis, MN) in accordance to the manufacturer’s instructions.

Rat peripheral blood mononuclear cells isolation. Blood from rats was diluted (1:1) in phosphate-buffered saline (PBS), pH 7.4, and this suspension was layered onto Histopaque-1077 and centrifuged for 30 min at 800 g and 4°C. Peripheral blood mononuclear cells (PBMCs) were collected from the interphase. PBMCs were then suspended in lysis solution for erythrocyte removal and washed twice with PBS. The PBMCs were maintained in RPMI-1640 medium supplemented with 10% of fetal bovine serum (FBS) (Thermo Fisher Scientific).

Rat splenocyte isolation. Ten weeks after LV radiofrequency surgery, the rats were anesthetized and their spleens were removed. The spleens were gently dissociated with autoclaved slides in a phosphate buffer solution. After complete dissociation, the homogenate was centrifuged at 300 g for 10 min at 4°C. The supernatant was discarded and the cell pellet was suspended in a lysis solution for 10 min on ice for erythrocyte removal. The cells were centrifuged at 300 g for 10 min at 4°C, and the cell pellet was suspended in PBS. Thereafter, the cells were centrifuged again at 300 g for 5 min at 4°C, resuspended, and maintained in RPMI-1640 medium supplemented with 10% FBS.

Determination of DPPIV concentration and activity. Plasma and heart DPPIV activity were determined spectrophotometrically by measuring the release of p-nitroaniline that resulted from the hydrolysis of glycylproline p-nitroanilide tosylate, as previously described (13). Heart DPPIV activity was normalized to total protein levels that were assessed using the Lowry method (27). DPPIV levels were evaluated in the culture medium by enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone, Houston, TX).

Assessment of proinflammatory markers in HF patients. All patients signed an informed consent form, and the Ethics Committee of
the Heart Institute of the University of São Paulo, São Paulo, Brazil, approved the study. Seventy-six HF patients from an ongoing inception cohort from the General Outpatient Clinic of the Heart Institute (InCor), University of São Paulo Medical School, were included in this study (Table 2). Subjects were enrolled in the study from 2005 to 2010. After enrollment, the serum samples were frozen at −80°C until analysis. The diagnosis of HF was based on previously published criteria (33), and the classification of HF etiology followed previous recommendations (37). Patients with symptomatic HF of varying etiology were eligible for enrollment into the cohort. We excluded patients with cardiomyopathy due to valvular heart disease that were candidates for conventional surgical treatment, such as valve repair or replacement, including patients with hypertrophic cardiomyopathy, chronic obstructive pulmonary disease, recent myocardial infarction, and/or unstable angina. Additionally, patients with severe renal or hepatic dysfunction, severe peripheral artery disease, recent myocardial infarction, or chronic kidney disease and a body mass index <25 were used as appropriate controls.

Statistical analysis. The data are expressed as the mean ± SE, unless otherwise specified. For comparisons between two groups, an unpaired t-test was used. If more than two groups were compared, the results were analyzed by one-way ANOVA followed by Bonferroni’s post hoc test. The correlation between DPPIV activity and inflammatory markers in patients was assessed by Pearson Correlation test. P < 0.05 was considered statistically significant.

RESULTS

Sitagliptin-induced cardioprotection in HF rats is associated with anti-inflammatory effects. Six weeks after myocardial injury in the HF or sham-operated models, the three groups of rats: sham (412 ± 8 g), HF (405 ± 4 g), and HF + iDPPIV (408 ± 9 g) exhibited similar mean body weights. Rats that were subjected to the LV ablation surgery displayed cardiac hypertrophy, pulmonary congestion, decreased ejection fraction, and increased LV end-diastolic pressure (Fig. 1, A–D), indicating heart failure development. Consistent with previous studies (13, 19), treatment with the DPPIV inhibitor sitagliptin was able to mitigate cardiac hypertrophy and dysfunction. In addition, HF rats exhibited increased levels of plasma and cardiac DPPIV activity (Fig. 1, E and F) and increased phosphorylation levels of p38 in the heart (Fig. 2A), which were attenuated by sitagliptin treatment (Figs. 1, E and F, and 2). Figure 2 also shows that the number of macrophages nearby the lesion of HF in rats was significantly increased compared with hearts from sham-operated rats and that treatment with the DPPIV inhibitor reduced those levels (Fig. 2B). Furthermore, the levels of the proinflammatory markers TNF-α, IL-1β, and IL-6 and as well as the chemokine CCL2 was increased in the hearts of HF rats, and sitagliptin significantly attenuated the

![Graphs](http://ajpheart.physiology.org/)

Fig. 3. The cardiac expression of inflammatory markers in sham, HF rats and HF rats treated with sitagliptin. The relative mRNA levels of TNF-α (A), IL-1β (B), CCL2 (C), IL-6 (D), and IL-10 (E) were determined by real-time RT-PCR in the hearts of sham, HF rats, and HF rats treated with sitagliptin (iDPPIV). Values are the means ± SE; n = 6 rats/group. *P < 0.05 vs. sham and #P < 0.05 vs. HF.

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abundance of most of these inflammatory markers with the exception of IL-6 (Fig. 3, A–D). The levels of the anti-inflammatory cytokine IL-10 did not differ among the three groups of rats (Fig. 3E).

M1 and M2 describe the two major phenotypes of activated macrophages. M1 macrophages usually function as key effector cells for the elimination of pathogens and Th1 responses. Conversely, M2 macrophages usually function in Th2 responses and tissue repair. In an attempt to characterize the heart macrophage phenotype in our experimental HF rats and whether DPPIV inhibition by sitagliptin could affect macrophage polarization, we evaluated the levels of cardiac M1 and M2 markers (Fig. 4) in sham, HF rats, and HF rats treated with sitagliptin. We found that, although the levels of the M2 marker arginase-1 did not differ among the groups (Fig. 4A), HF rats exhibited increased levels of the M1 marker iNOS (Fig. 4B), and a lower M2/M1 ratio than that of the sham was observed (Fig. 4C). Moreover, the expression of Irf5 (Fig. 4D), which is usually associated with M1 polarization (22), was also increased in HF. Finally, treatment with sitagliptin significantly prevented this proinflammatory profile by reducing the expression of M1 markers and normalizing the M2/M1 ratio.

Sitagliptin-induced cardioprotection in HF rats is associated with improved cardiac perfusion. Because M2 macrophages play a role in tissue repair and DPPIV is known to cleave and inactivate CXCL12, an important chemokine associated with angiogenesis (50), we next evaluated whether chronic treatment with sitagliptin would improve cardiac perfusion and angiogenesis. Cardiac perfusion was assessed by micro-SPECT. As shown in Fig. 5, A and B, the rats subjected to myocardial injury displayed a significant decrease in cardiac perfusion compared with the sham-operated rats. In addition, the rats treated with sitagliptin exhibited a significant increase in cardiac perfusion compared with the HF animals. Next, ELISA and real-time RT-PCR were used to analyze the levels of cardiac vascular endothelial growth factor (VEGF) and CXCL12, respectively. No statistically significant differences were found in the levels of the cardiac CXCL12 (Fig. 5C) and VEGF (Fig. 5D) among the three groups of rats.

Fig. 4. Cardiac expression of M1 and M2 macrophage markers in sham, HF rats and HF rats treated with sitagliptin. Relative mRNA levels of Arg-1 (A), inducible nitric oxide synthase (iNOS; B); Arg-1/iNOS (C) ratio, and interferon regulatory factor 5 (IRF5; D) were determined by real-time RT-PCR in the hearts of sham, HF rats, and HF rats treated with sitagliptin (iDPPIV). The values are the means ± SE; n = 6 rats/group. *P < 0.05 vs. sham and #P < 0.05 vs. HF.
press GLP-1R (Fig. 6D). By costaining heart sections isolated from the three experimental groups of rats, we were able to demonstrate that GLP-1R colocalizes with the macrophage marker CD68, suggesting that GLP-1 may act directly on those cells. The colocalization of GLP-1R with CD68 in the heart was mainly observed in HF rats, since these animals possessed a higher number of macrophages in the heart compared with either sham or HF rats treated with sitagliptin (Fig. 2B).

Next, we evaluated the effects of the GLP-1R agonist exendin-4 (Ex-4), on isolated and cultured rat peritoneal macrophages. In line with our hypothesis, we found that treatment with Ex-4 significantly attenuated LPS-mediated TNF-α, IL-1β, and IL-6 secretion (Fig. 6, E–G). Interestingly, although we detected the presence of GLP-1R by immunofluorescence (Fig. 6D) and demonstrated the functional expression of this receptor (Fig. 6, E–G), we were unable to find colocalization between CD68 and DPPIV in the hearts of HF rats (data not shown).

The correlation between DPPIV and inflammatory markers in HF patients. Given the anti-inflammatory effects of DPPIV inhibition and Ex-4 in vivo and in vitro, respectively, and that inflammation plays a key role in the pathophysiology of HF, we sought to examine the levels of inflammatory markers and whether DPPIV activity correlates with those markers in HF patients.
patients. As shown in Fig. 7, A and B, HF patients exhibited higher serum levels of both TNF-α (7.43 ± 0.41 vs. 3.55 ± 0.24, P < 0.001) and CCL2 (198 ± 13 vs. 120 ± 11, P < 0.001) than control subjects.

Next, we assessed if serum DPPIV activity correlates with the plasma levels of TNF-α and CCL2 in HF patients. As shown in Fig. 7, C and D, DPPIV activity did not correlate with TNF-α levels (r = −0.15; P = 0.20). Conversely, a significant
correlation was found between the levels of CCL2 ($r = 0.32$; $P = 0.004$) and the serum activity of DPPIV in HF patients.

DPPIV release is increased in splenocytes from HF rats. Taking into account that 1) CCL2 plays a fundamental role in leukocyte recruitment, 2) DPPIV activity is increased in HF patients and positively correlates with this chemokine, and 3) DPPIV is expressed in the cell surface of leukocytes, we hypothesized that circulating mononuclear cells (PBMCNs) may be involved in increasing the circulating levels of DPPIV in HF. To this end, PBMCNs from sham and HF rats were isolated and cultured for 5 days, and the levels of DPPIV were evaluated in the culture cell medium. As shown in Fig. 8A, no difference was found between the levels of DPPIV released to the media by PBMCNs derived from HF rats compared with those of sham rats.

Compelling evidence from recent studies suggests that the spleen contributes to chronic inflammation and immune cell-mediated injury in HF (20, 25, 48, 52). Thus, under conditions similar to those of the PBMCN experiments, DPPIV abundance was measured in the cultured medium of isolated splenocytes from HF and sham-operated rats. As illustrated in Fig. 8B, HF splenocytes released ~70% more DPPIV than splenocytes derived from sham rats. In addition to an increase release of DPPIV, HF splenocytes also release more IL-1$\beta$ (Fig. 8C) but not TNF-\(\alpha\) into the cell culture medium compared with sham rats (Fig. 8D).

**DISCUSSION**

This study demonstrates that DPPIV inhibition by sitagliptin significantly attenuates cardiac inflammation and improves cardiac perfusion in an experimental model of HF. Moreover, we demonstrate that not only that HF rats exhibit increased levels of inflammatory markers in the heart but also that DPPIV released into the culture medium is higher in splenocytes derived from HF rats than sham-operated rats. Additionally, we showed that serum DPPIV activity correlates with the proinflammatory chemokine CCL2 in HF patients.

Sitagliptin-induced cardioprotection after myocardial injury in rats appears to be glucose independent and, at least in part, due to its anti-inflammatory effects. This conclusion was based on the findings that DPPIV inhibition did not change blood glucose levels and was associated with attenuated infiltration of macrophages in the heart after injury and reduced expression of several inflammatory markers such as p38 phosphorylation, TNF-\(\alpha\), IL-1$\beta$, and the monocyte chemoattractant chemokine CCL2. Of note, different groups have shown that DPPIV inhibition might attenuate inflammation in animal models of inflammatory diseases. Shah et al. (45) previously showed that long-term DPPIV inhibition reduces inflammation in a mouse model of experimental atherosclerosis. Moreover, glucose-independent improvement of vascular dysfunction and inflammation was achieved in experimental sepsis after treatment
Because M2 macrophages are usually associated with tissue repair and angiogenesis, we evaluated cardiac perfusion and the levels of angiogenic factors in sitagliptin-treated and nontreated rats. In contrast with a previous study in which DPPIV inhibition increased the levels of CXCL12 and improved vascular density after myocardial infarction (57), we did not find any difference in mRNA expression of this chemokine or the content of VEGF in the hearts of sitagliptin-treated rats compared with HF rats. Nevertheless, our findings indicate that DPPIV inhibition increases heart perfusion. There are several explanations for this apparent paradox. One explanation is the fact that the expression of CXCL12 usually increases after an acute ischemic event such as a myocardial infarction and subsequently returns to basal levels (57). Thus it is possible that after 6 wk of cardiac injury CXCL12 levels are already normalized. Moreover, several DPPIV substrates possess vasodilator actions and might contribute to the increase in cardiac perfusion in the absence of angiogenesis. It is well established that activation of GLP-1R induces vasorelaxation in a variety of vascular beds including conductance and resistant vessels (6, 7). Moreover, BNPs, which is the truncated form of BNP after DPPIV cleavage, lacks vasodilator and natriuretic actions (8). Thus treatment with sitagliptin might increase the half-life of these vasodilator peptides to increase cardiac perfusion.

Notably, BNP has also been implicated in cardiac inflammation. In fact, BNP is upregulated at the transcriptional and translational levels by proinflammatory cytokines such as TNF-α and IL-1β in a p38-dependent manner in ventricular cardiomyocytes. Interestingly, unlike TNF-α and IL-1β, BNP regulation/secretion was unchanged after IL-6 stimulation (29). These data are in accordance with the data obtained from our present and previous studies because, with the exception of IL-6, DPPIV inhibition is capable of reducing these proinflammatory markers in the heart and circulating levels of total BNP in HF rats (13). Interestingly, it was previously shown that BNP inhibits CCL2-induced monocyte migration in vitro (16). It remains to be established whether the truncated form of BNP, BNPs, also lacks antiinflammatory effects.

Increased levels of DPPIV have been associated with poor outcomes in HF animals and patients (13, 26, 46). Several mechanisms may be responsible for this poor prognosis because several cardioprotective peptides are inactivated by DPPIV. However, the fact that we found a positive correlation between DPPIV and the monocyte chemoattractant protein CCL2 may shed light on the role of DPPIV on the pathophysiology of this syndrome. Several clinical studies have shown that high levels of circulating CCL2, IL-6, and TNF-α or their receptors significantly correlate with the New York Heart Association functional class and poorer prognosis in HF (9). Conversely, anti-inflammatory therapies fail to achieve positive outcomes in HF patients (14). CCL2 plays a crucial role in leukocyte recruitment to injured and inflamed tissues such as the failing heart. High levels of CCL2 increases the number of inflammatory cells, and this, combined with an increased secretion of proinflammatory mediators such as TNF-α, IL-6, IL-1, and Toll-like receptor ligands, may promote cardiomyocyte cell death and cardiac remodeling and modulate fibroblast phenotypes, which all contribute to decreased cardiac function (14).

The source of the inflammatory cells that migrate into the heart might be diverse and include not only the bone marrow with linagliptin (23). Thus understanding the connection between DPPIV and inflammation may lead to new therapeutic targets for diseases that have inflammation as an important component. Interestingly, treatment with sitagliptin reduces inflammatory biomarkers such as TNF-α and C-reactive protein in humans with type 2 diabetes (32, 38, 41). However, because improved glycemic control was also achieved in those cases, a glucose-dependent effect cannot be ruled out.

The molecular mechanisms underlying the anti-inflammatory effects of sitagliptin are complex because DPPIV is capable of inactivating several peptides. Our current study suggests that GLP-1 may be involved in mediating the cardioprotective and anti-inflammatory effects of sitagliptin. In this regard, we found that DPPIV inhibition increases plasma levels of active GLP-1, GLP-1R is expressed in heart macrophages and treatment with the GLP-1R agonist exendin-4 significantly attenuates the macrophage LPS-mediated secretion of TNF-α, IL-1β, and IL-6. Interestingly, Shiraiishi et al. (47) suggested that GLP-1 induces M2 polarization of human macrophages via STAT3 activation. Although we did not measure the levels of STAT3 phosphorylation, we did find a decrease in M1 markers such as iNOS and If5 in the hearts of sitagliptin-treated rats compared with untreated HF rats. Thus one may conclude that the GLP-1R pathway may play an important role in cardiac protection by reducing cytokine secretion and modulating the M2/M1 balance.
but also extramedullary organs such as the spleen. Indeed, it has been recently reported that the spleen does not only play a role in acute inflammation (25, 48, 52) but also plays a role in chronic inflammation and HF progression (20). In this regard, we found that splenocytes derived from HF rats release higher levels of IL-1β and 70% more DPPIV into the culture medium than splenocytes derived from healthy rats. Conversely, the same pattern was not observed with PBMCNs, suggesting that DPPIV release may occur before spleen emigration or through a distinct cell population that is represented in the spleen but not circulating mononuclear cells. Because the spleen mostly comprises myeloid cells and lymphocytes and DPPIV expression in dendritic/macrophages is usually very low (24, 53), we might speculate that splenic lymphocytes may contribute to the observed increase in plasma DPPIV in HF. Thus the spleen most likely has an important role in the progression of HF because it may aggravate cardiac function by increasing the output of inflammatory cells into the heart and by increasing the levels of soluble DPPIV, which in turn inactivates several cardio-, vaso-, and renoprotective peptides namely GLP-1, BNP, and CXCL12. Of note, plasma DPPIV is also increased in other diseases such as obesity and hypertension (28, 36), and recent studies have shown that the spleen has an important role in the pathophysiology of these disorders (39, 55). It is, therefore, plausible to hypothesize that the immune system and possibly the spleen may be responsible for the increased levels of soluble DPPIV in those illnesses. In line with this hypothesis, studies have shown not only that plasma DPPIV is increased but also that DPPIV inhibition is able to reduce inflammation and end-organ damage in obesity and hypertension (31, 35).

Despite the extensive experimental evidence documenting the cardiovascular benefits of DPPIV inhibition, the cardioprotective effects of gliptins in the clinical setting have yet to be proven. In the past few years, the results of three, large, prospective clinical studies evaluating the cardiovascular outcomes in type 2 diabetic patients treated with DPPIV inhibitors were published (16, 42, 54). The results from the SAVOR-TIMI 53 trial (43) raised the concern that DPPIV inhibitors could increase the risk of HF exacerbation. In particular, the authors found that patients assigned to the saxagliptin group had a higher hospitalization admission rate for HF than placebo, especially in those patients who displayed a higher overall risk of HF (e.g., history of HF, chronic kidney disease or elevated baseline levels of NH2-terminal pro-brain natriuretic peptide) (44). Conversely, the subsequent other two trials found no differences between the rates of hospitalization for HF in type 2 diabetic patients treated with alogliptin (EXAMINE) or sitagliptin (TECOS) and those of patients treated with placebo (17, 54, 56), indicating that the increased risk for HF exacerbation observed in the SAVOR-TIMI 53 trial does not constitute a drug class effect. It is important to emphasize that although the aforementioned trials either demonstrated disappointing or neutral results, the duration of these studies (weighted average 2.3 yr) is not sufficient to evaluate whether gliptins may or may not exert cardioprotection.

Taken together, our data suggest that the cardioprotective effects of DPPIV inhibition in HF may be due to increased bioavailability of DPPIV substrates with anti-inflammatory actions. Moreover, increased cytokine production in chronic HF appears to be paralleled by increased DPPIV release into the extracellular milieu. Therefore, it is tempting to speculate that an interplay may exist between DPPIV and inflammation in HF.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


