Genetic Deletion or Pharmacological Inhibition of Dipeptidyl Peptidase-4 Improves Cardiovascular Outcomes After Myocardial Infarction in Mice

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OBJECTIVE—Glucagon-like peptide-1 (7-36)amide (GLP-1) is cleaved by dipeptidyl peptidase-4 (DPP-4) to GLP-1 (9-36)amide. We examined whether chemical inhibition or genetic elimination of DPP-4 activity affects cardiovascular function in normoglycemic and diabetic mice after experimental myocardial infarction.

RESEARCH DESIGN AND METHODS—Cardiac structure and function was assessed by hemodynamic monitoring and echocardiography in DPP-4 knockout (Dpp4−/−) mice versus wild-type (Dpp4+/+) littermate controls and after sitagliptin versus treatment with metformin were ascertained after experimental MI in a high-fat diet–streptozotocin model of murine diabetes. Functional recovery from ischemia-reperfusion (I/R) injury was measured in isolated hearts from Dpp4−/− versus Dpp4+/+ litters and from normoglycemic wild-type (WT) mice treated with sitagliptin or metformin. Cardioprotective signaling in the murine heart was examined by RT-PCR and Western blot analyses.

RESULTS—Dpp4−/− mice exhibited normal indexes of cardiac structure and function. Survival post-MI was modestly improved in normoglycemic Dpp4−/− mice. Increased cardiac expression of phosphorylated AKT (pAKT), pGSK3β, and atrial natriuretic peptide (ANP) was detected in the nonischemic Dpp4−/− heart, and HO-1, ANP, and pGSK3β proteins were induced in nonischemic hearts from diabetic mice treated with sitagliptin or metformin. Sitagliptin and metformin treatment of wild-type diabetic mice reduced mortality after myocardial infarction. Sitagliptin improved functional recovery after I/R injury ex vivo in WT mice with similar protection from I/R injury also manifest in hearts from Dpp4−/− versus Dpp4+/+ mice.

CONCLUSIONS—Genetic disruption or chemical inhibition of DPP-4 does not impair cardiovascular function in the normoglycemic or diabetic mouse heart. Diabetes 59:1063–1073, 2010

Type 2 diabetes is associated with an increased risk of cardiovascular disease, hence there is considerable interest in strategies that reduce cardiovascular morbidity and mortality in diabetic subjects. Although aggressive treatment of blood pressure and dyslipidemia reduces cardiovascular events in both nondiabetic and diabetic patients, whether reduction in blood glucose alone reduces cardiac events in subjects with established diabetes remains controversial (1). Moreover, pharmacotherapy of diabetes using agents with unique antidiabetic mechanisms may be associated with differential and occasionally unexpected adverse effects on cardiovascular outcomes, independent of effects on glucose control (2,3). Therefore, a detailed understanding of the unique cardiovascular benefits and risks of each antidiabetic drug used to treat diabetes seems prudent.

The two most recently approved drug categories for the treatment of type 2 diabetes, GLP-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors, exert their antidiabetic actions largely through potentiation of GLP-1R activation (4). Because these agents have only been used clinically for several years, there are scant data on cardiovascular outcomes associated with these incretin-based therapies. GLP-1 improves endothelial function in subjects with type 2 diabetes (5), and transient GLP-1 administration improves cardiovascular outcomes in subjects with myocardial infarction (MI) or congestive heart failure (CHF) (6,7). Moreover, preclinical data demonstrate that GLP-1 is cardioprotective when administered before the induction of ischemia (8–10). Furthermore, therapy with the GLP-1R agonists exenatide and liraglutide is associated with blood pressure reduction in the majority of treated subjects (11,12). Hence, although limited, the available data support the hypothesis that antidiabetic therapy with GLP-1 may be associated with beneficial effects on cardiovascular outcomes. Nevertheless, as GLP-1R agonists may produce weight loss (13), the extent to which the salutary effects of GLP-1R activation on the cardiovascular system in vivo reflect the beneficial consequences of weight loss remains unclear.

In contrast, much less is known about the cardiovascular biology of DPP-4. Unlike therapy with GLP-1R agonists, the use of sitagliptin, saxagliptin, or vildagliptin has not been associated with weight loss or sustained improvement in lipid profiles (4). Moreover, inhibition of DPP-4 enzyme activity modulates the activity of cardioactive peptides such as brain natriuretic peptide, neuropeptide Y, and stromal cell–derived factor-1 (SDF-1) (14) via non–GLP-1 mechanisms of action. More recently, GLP-1 (9-36), a peptide metabolite derived from native GLP-1 (7-36)amide, exhibits cardioprotective properties in experimental models of myocardial infarction (MI) (7,15). These observations support the potential for GLP-1 to modulate the rehabilitation of myocardial infarction (MI) (16–19).

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DPP-4 AND CARDIOVASCULAR OUTCOMES

FIG. 1. Dpp4−/− mice exhibit increased survival after myocardial ischemic injury. A: Heart weight (HW)–to–body weight (BW) ratios in 12-week-old Dpp4+/+ versus Dpp4−/− mice not exposed to LAD ligation. B: Experimental scheme for analysis of normoglycemic Dpp4+/+ and Dpp4−/− mice on a regular diet. C: MI was induced by permanent coronary artery ligation (LAD) and survival was assessed in male and female 12-week-old Dpp4−/− and Dpp4+/+ mice over the subsequent 4 weeks. D: Hypertrophy is observed in Dpp4+/+ and Dpp4−/− mice after MI. E: Infarct size was determined through quantitative histological analysis of hearts from male and female Dpp4+/+ and Dpp4−/− mice. *P < 0.05; numbers in brackets correspond to number of male and female mice (combined) per treatment.

36)amide after cleavage by DPP-4, has been shown to exert cardioprotective actions in rodents (15,16) and improve cardiovascular function in dogs with CHF (17). Accordingly, to delineate the importance of DPP-4 for cardiovascular biology in vivo, we studied cardiovascular function in Dpp4−/− mice in vivo, in isolated perfused Dpp4+/+ and Dpp4−/− hearts ex vivo, and in wild-type diabetic mice subjected to experimental MI and treated with the DPP-4 inhibitor sitagliptin.

RESEARCH DESIGN AND METHODS

Animal models and drug treatments. Experimental procedures adhered to approved protocols of the University Health Network and Mt. Sinai Hospital Animal Care Committees. Mice were housed under pathogen-free conditions in micro-isolator cages and maintained on a 12-h light (0700)/dark (1900) cycle with access to standard rodent food and water ad libitum, except where noted. All experiments used age- and sex-matched littermates. Dpp4−/− mice were inbred on the C57BL/6 background (18). Experimental animals were noted. All experiments used age- and sex-matched littermates. Dpp4−/− mice were inbred on the C57BL/6 background (18). Experimental animals were

sodium citrate, pH 5.5. Mice were then randomized to treatment with either HFD alone, HFD plus a DPP-4 inhibitor (sitagliptin, 250 mg · kg−1 · day−1) (19), or HFD plus metformin (450 mg · kg−1 · day−1). The dose of metformin was chosen based on related studies (20) and following pilot studies demonstrating optimal antidiabetic actions without significant effects on food intake or body weight. This dose of sitagliptin does not affect food intake, yet results in 90% inhibition of DPP-4 (19,21). Sitagliptin and metformin were supplied by Merck Research Labs (Rahway, NJ).

For RNA and protein analyses by real-time quantitative PCR and Western blot, respectively, heart tissue was obtained from separate groups of normoglycemic Dpp4+/+ or Dpp4−/− mice fed regular food, or wild-type C57BL/6 mice exposed to HFD for 4 weeks, given a single dose of STZ (75 mg/kg), and then treated with either HFD alone, or HFD plus either sitagliptin, 250 mg · kg−1 · day−1 or HFD plus metformin (450 mg · kg−1 · day−1). The dose of metformin was chosen based on related studies (20) and following pilot studies demonstrating optimal antidiabetic actions without significant effects on food intake or body weight. This dose of sitagliptin does not affect food intake, yet results in 90% inhibition of DPP-4 (19,21). Sitagliptin and metformin were supplied by Merck Research Labs (Rahway, NJ).

Metabolic measurements. Oral glucose tolerance tests were performed in sitagliptin- or metformin-treated wild-type C57BL/6 mice and untreated controls. Mice were fasted for 16 h and administered glucose (1.5 mg/g) via oral gavage. AIC and blood glucose levels were measured on whole blood using the DCA 2000+ Analyzer and a Glucometer (both Bayer, Toronto, ON, Canada). GLP-1 (7-36)amide levels were measured by a Meso Scale Discovery
Coronary artery ligation. Experimental MI was induced by left anterior descending (LAD) artery ligation as described (22). Briefly, 12-week-old male and female Dpp4+/− and Dpp4−/− mice were anesthetized using 1% isoflurane, intubated in positive end-expiratory pressure, and the thoracotomy was closed. Animals were kept on the ventilator until awake. Sham operation differed only in that the 7-0 suture was passed under the coronary artery and then removed.

Ultrasonic biomicroscopy in mice after LAD artery ligation. Male C57BL/6 mice, 10 weeks old, from Tacnic (Germantown, NY) were housed under pathogen-free conditions in micro-isolator cages and maintained on a control diet (Research Diets) or a diet containing sitagliptin (250 mg/kg) for 1 week before LAD ligation. High-frequency ultrasound imaging was carried out on day 4 post-MI. Mice were killed on day 5 after coronary artery ligation, and hearts were collected for RNA and protein analyses.

Isolated heart preparations. After administration of heparin (1,000 IU/kg s.c.) and sodium pentobarbital (200 mg/kg i.p.), hearts were excised, cannulated through the aorta, and perfused at 80 mmHg in a Langendorff apparatus with gassed (95% O2, 5% CO2) Krebs-Henseleit buffer (mM/l: 118 NaCl, 4.7 KCl, 11 glucose, 1.2 MgSO4, 25 NaHCO3, 1.2 KH2PO4, and 25 CaCl2) maintained at 37°C (Harvard Apparatus, Holliston, MA) as described (15). For measurement of isovolumetric pressures, a small plastic balloon was inflated in the LV via a small left atrial incision. Both end systolic (LVSEP) and end diastolic (LVEDP) pressures were monitored, the latter maintained between 4 and 8 mmHg throughout the experiment. LV developed pressure (LVDP) was calculated as a% of total LV area.

Statistical analysis. Data were analyzed using ANOVA followed by Bonferroni’s post hoc tests. P < 0.05 was considered statistically significant.

RESULTS

Cardiac structure and function in Dpp4−/− mice. We first verified that Dpp4−/− mice used in our studies retained the phenotype of improved glucose tolerance and reduced DPP-4 activity as originally described (18). Consistent with previous findings, oral glucose tolerance was improved and plasma DPP-4 activity was markedly reduced in Dpp4−/− mice (supplemental Fig. 1A–C, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0955/DC1). Heart weights did not differ between 12-week-old sex-matched Dpp4+/− and Dpp4−/− mice (Fig. 1A, P = NS). High-resolution echocardiography did not detect any differences in LV wall thicknesses, LV end systolic and end diastolic dimensions, mitral and aortic flow velocities, LV systolic and diastolic areas, LV outflow tract diameter, aortic ejection time, left atrial size, or fractional shortening between Dpp4+/− and Dpp4−/− mice (Table 1, NS for all comparisons). Hence, genetic disruption of the Dpp4 gene in mice is not associated with baseline abnormalities in cardiac structure or function.

Myocardial infarction outcomes in normoglycemic Dpp4−/− mice. To determine whether disruption of the Dpp4 gene modifies the response to cardiac injury, we induced MI in nondiabetic 12-week-old male and female Dpp4−/− and Dpp4+/− mice via permanent surgical LAD ligation (Fig. 1B). At the predefined end point of 4 weeks post-MI, Dpp4−/− mice exhibited an ~20% absolute increase in survival compared with Dpp4+/− littermate controls (Fig. 1C). Post-MI, the hearts of Dpp4+/− and

Table 1

Echocardiographic defined and functional parameters in Dpp4+/− and Dpp4−/− mice

| Parameter | Dpp4+/− | Dpp4−/− |
|-----------|---------|---------|
| n         | 5       | 5       |
| Aortic flow |         |         |
| Aortic output flow (cm/s) | 72.8 ± 4.4 | 63.6 ± 2.9 |
| Aortic valve ejection time (ms) | 68.5 ± 3.9 | 69.6 ± 3.6 |
| Mitral flow |         |         |
| Peak E velocity (cm/s) | 51.7 ± 2.3 | 47.7 ± 5.0 |
| Deceleration time (ms) | 42.5 ± 3.5 | 47.1 ± 1.3 |
| LV chamber dimensions by M-mode |         |         |
| Left atrium size (mm) | 1.74 ± 0.06 | 1.66 ± 0.03 |
| LV end diastolic diameter (mm) | 3.85 ± 0.22 | 3.87 ± 0.09 |
| LV end systolic diameter (mm) | 2.45 ± 0.18 | 2.62 ± 0.08 |
| LV outflow tract diameter (mm) | 1.17 ± 0.03 | 1.14 ± 0.02 |
| Anterior wall thickness (mm) | 0.74 ± 0.30 | 0.76 ± 0.01 |
| Posterior wall thickness (mm) | 0.77 ± 0.04 | 0.74 ± 0.02 |
| Fractional shortening (%) | 36.42 ± 2.17 | 32.85 ± 1.04 |

Data are means ± SE. Genetic elimination of DPP-4 activity is not associated with abnormalities in parameters of cardiovascular function. Transthoracic echocardiographic studies were carried out in male 12-week-old Dpp4+/− and Dpp4−/− mice. Mice were lightly anesthetized using 3% isoflurane/97% oxygen. Two-dimensional and M-mode echocardiography, as well as pulsed Doppler analyses, were performed by a blinded observer using a Hewlett-Packard 5500 ultrasound device (Hewlett-Packard, Palo Alto, CA) and a 12-MHz phased array and 15-MHz Doppler probes. Three M-mode recordings of end systolic and end diastolic LV internal diameters and end diastolic LV posterior wall thicknesses were made. A single mean measurement was then calculated for each mouse. Analysis of data was carried out as previously described (44,45).
Dpp4+/+ and Dpp4−/− mice underwent similar compensatory hypertrophy (Fig. 1D). Although infarct size was reduced in Dpp4−/− mice, this difference was not statistically significant (Fig. 1E).

To explore mechanisms mediating the increased survival of Dpp4−/− mice post-MI, we analyzed cardiac mRNA and protein levels of known cardioprotective genes. Normoglycemic nonischemic Dpp4−/− mice exhibited small but nonsignificant increases in Akt1, Gsk3β, Ppara, PI3K, and Ho1 transcripts (Fig. 2B–F). Moreover, hearts from Dpp4−/− mice contained higher levels of phosphorylated AKT (pAKT), pGSK3β, and ANP (Fig. 2G–I), proteins known to be regulated by GLP-1R agonists (10) and associated with cardioprotection in vivo (24–27).

**Treatment of diabetic mice with metformin or sitagliptin pre- and post-MI.** Because Dpp4−/− mice are resistant to the development of STZ-induced diabetes (28), we assessed whether reduction of DPP-4 activity is car-
dioprotective in diabetic Dpp4+/− mice. Wild-type mice were placed on HFD (45% fat) for 4 weeks (A). At the start of week 5, mice were injected with a single dose of STZ (75 mg/kg) and then randomized into three treatment groups: 1) HFD/STZ alone, 2) HFD/STZ + sitagliptin (250 mg/kg), or 3) HFD/STZ + metformin (450 mg/kg) for an additional 8 weeks. At week 12, mice underwent LAD ligation or control sham surgery. At week 16, surviving mice were killed and infarct size was measured. Levels of random fed blood glucose (B) and A1C (C) were significantly reduced in mice treated with sitagliptin or metformin. Oral glucose tolerance (D and E) was significantly improved in sitagliptin-treated (n = 29) or metformin-treated (n = 23) mice compared with HFD/STZ alone, sitagliptin, or metformin (n = 22–23 per treatment). Plasma active GLP-1 is increased in mice treated with sitagliptin or metformin (n = 14 per treatment) (G). *P < 0.05, ***P < 0.001 vs. the untreated HFD/STZ group. AUC, area under the curve.

FIG. 3. Sitagliptin and metformin reduce blood glucose levels and increase plasma GLP-1 (7-36)amide levels in diabetic mice. Male C57BL/6 mice were placed on HFD (45% fat) for 4 weeks (A). At the start of week 5, mice were injected with a single dose of STZ (75 mg/kg) and then randomized into three treatment groups: 1) HFD/STZ alone, 2) HFD/STZ + sitagliptin (250 mg/kg), or 3) HFD/STZ + metformin (450 mg/kg) for an additional 8 weeks. At week 12, mice underwent LAD ligation or control sham surgery. At week 16, surviving mice were killed and infarct size was measured. Levels of random fed blood glucose (B) and A1C (C) were significantly reduced in mice treated with sitagliptin or metformin. Oral glucose tolerance (D and E) was significantly improved in sitagliptin-treated (n = 29) or metformin-treated (n = 23) mice compared with HFD/STZ alone, sitagliptin, or metformin (n = 22–23 per treatment). Plasma active GLP-1 is increased in mice treated with sitagliptin or metformin (n = 14 per treatment) (G). *P < 0.05, ***P < 0.001 vs. the untreated HFD/STZ group. AUC, area under the curve.

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gliptin or metformin, compared to mice on HFD/STZ alone (Fig. 4A). A significant increase in heart-to-body weight ratios post-MI was observed only in diabetic mice treated with metformin (Fig. 4B). No differences in infarct size were observed between the three groups (Fig. 4C).

To identify potential mechanism(s) underlying improved survival post-MI in sitagliptin- and metformin-treated diabetic mice, we assessed cardiac mRNA and protein levels of candidate pro-survival genes in separate groups of diabetic animals treated for 1 week with either sitagliptin, metformin, or the GLP-1R agonist, liraglutide. No significant changes were detected in mRNA levels of PI3k, Akt, Ho1, Mmp9, and Ppara after treatment with these antidiabetic agents (Fig. 5B–F). In contrast, sitagliptin, metformin, and liraglutide increased expression of ANP (Fig. 5G). Sitagliptin and liraglutide, but not metformin, activated the prosurvival kinase AKT (Fig. 5H), whereas all three drugs increased levels of HO-1 (Fig. 5J). A modest but nonsignificant increase in levels of phospho-GSK3β was also observed with all three antidiabetic agents (Fig. 5J).

We next examined whether metformin or sitagliptin treatment of HFD-fed mice produced changes in gene and protein expression in the mouse heart after LAD ligation (Fig. 6A). Levels of Ho1 and Gsk3β RNA transcripts were modestly increased (Fig. 6D and E), whereas Akt1, PI3k, and Ppara RNA transcripts were significantly increased in the post-ischemic heart after metformin treatment (Fig. 6B, C, and F). In contrast, sitagliptin treatment was not associated with significant changes in levels of cardioprotective mRNA transcripts post-MI (Fig. 6D–F). Neither metformin nor sitagliptin treatment produced detectable changes in levels of AKT, ANP, GSK3β, or HO-1 proteins assessed at day 5 post-MI (Fig. 6G–J). Similarly, although heart rate was increased in metformin-treated mice, we did not detect other significant differences in parameters of cardiac function in sitagliptin-versus metformin-treated mice after myocardial infarction (Table 2).

Ischemia-reperfusion injury and metformin versus DPP-4 inhibition in normoglycemic mouse hearts. To determine whether cardioprotective actions of sitagliptin are observed in the normoglycemic murine heart, we acutely administered sitagliptin, metformin, or saline (PBS) to nondiabetic wild-type mice in vivo before assessing recovery of LVDP after I/R injury to their hearts ex vivo (15). Parallel experiments included I/R injury in hearts from normoglycemic Dpp4−/− and Dpp4+/+ animals, as well as testing cardioprotective actions of an acute ex vivo sitagliptin infusion (20 min) versus placebo (PBS) immediately before wild-type hearts undergoing I/R injury (Fig. 7A).

Acute administration of metformin or sitagliptin in vivo improved recovery from subsequent I/R injury in normoglycemic mice (Fig. 7B). Recovery of LVDP was also greater in Dpp4−/− hearts versus Dpp4+/+ littermate controls (Fig. 7B). By contrast, sitagliptin administered to the coronary circulation ex vivo (and immediately before I/R injury) exerted no direct cardioprotective actions in isolated mouse hearts (Fig. 7C). Taken together, these data show that the cardioprotective effects of genetic or pharmacological inhibition of DPP-4 activity are not strictly glucose dependent and depend on one or more DPP-4-dependent actions in vivo.

DISCUSSION

Analysis of the cardiovascular profile of antidiabetic agents involves ascertainment of the effects of each drug on the myocardium and endothelium, and on secondary risk factors such as control of blood pressure and cholesterol. Although preclinical studies may be useful in generating hypotheses about the putative cardiovascular actions of different drug classes, the results of subsequent clinical studies have not always been concordant with predictions made from preclinical analyses. For example, although both thiazolidinediones, pioglitazone and rosiglitazone, exert beneficial effects on inflammation and endothelial function (29), pioglitazone, but not rosiglitazone, is associated with reduced cardiovascular events in human studies (3,30). Similarly, although data from both preclinical (31) and clinical studies (32) suggests that metformin therapy may be cardioprotective, the mechanisms through which metformin therapy is associated with cardioprotect-
FIG. 5. Treatment of diabetic C57BL/6 mice with sitagliptin, metformin, or liraglutide leads to increased expression of cardioprotective proteins. Diabetes was induced in HFD-fed STZ-treated WT C57Bl/6 mice (A). Levels of mRNA transcripts in hearts from mice were treated with HFD/STZ alone, or HFD/STZ plus sitagliptin, metformin, or liraglutide, for 1 week. Relative levels of PI3k (B), Akt1 (C), Ho1 (D), Mmp9 (E), and Pparα (F) were assessed by quantitative real-time PCR and normalized to levels of β-actin transcripts in the same samples, n = 6 per group. Western blot analysis is shown for ANP (G), AKT (H), HO-1 (I), and pGSK3β (J) using heart extracts from wild-type mice on HFD/STZ alone, or HFD/STZ plus either sitagliptin, metformin, or liraglutide. *P < 0.05, ***P < 0.001, n = 3 per treatment.
FIG. 6. Gene and protein expression after LAD occlusion in hearts from wild-type mice treated with metformin or sitagliptin. Nondiabetic 10-week-old mice were treated with sitagliptin or metformin for 1 week (A), and cardiac levels of mRNA transcripts for Akt1 (B), PI3k (C), Ho1 (D), Gsk3β (E), and Pparα (F) were determined by real-time PCR, normalized to the values of Gapdh mRNA transcripts in the same sample. Western blot analysis was used to ascertain levels of pAKT (G), ANP (H), pGSK3β (I), and HO-1 (J) 5 days after LAD ligation. HSP90 was used as an internal control protein. ***P < 0.001, n = 5 mice per group.
Aortic flow

Dpp4 improvements in survival after experimental MI in the present study. Zaruba et al. (35) also observed modest such as GLP-1 (18) or SDF-1 (35), cannot be inferred from cardiomyocytes or blood vessels, or indirectly due to the ligation is directly due to loss of DPP-4 activity per se in experimental MI. Whether the increased survival after LAD inhibition remain poorly understood. Moreover, sulfonylureas have been associated with increased rates of death from cardiovascular disease in some (33) but not all studies (32).

We have now investigated the cardiovascular consequences arising from genetic elimination or pharmacological inhibition of DPP-4 activity. DPP-4 has three major functions; adenosine deaminase binding, peptidase activity, and extracellular matrix binding, all of which potentially influence the activity of the immune and/or endocrine systems (34). Although DPP-4 cleaves and inactivates several cardioactive peptides, including neuropeptide Y, brain natriuretic peptide, SDF-1, and GLP-1, there is little information on the cardiovascular consequences of reduced or absent DPP-4 activity. Normoglycemic Dpp4−/− mice exhibit normal cardiac structure and function in the basal state, yet increased survival after experimental MI. Whether the increased survival after LAD ligation is directly due to loss of DPP-4 activity per se in cardiomyocytes or blood vessels, or indirectly due to the subsequent upregulation of cardioprotective molecules such as GLP-1 (18) or SDF-1 (35), cannot be inferred from the present study. Zaruba et al. (35) also observed modest improvements in survival after experimental MI in Dpp4−/− mice or in WT mice treated with a DPP-4 inhibitor, and more robust improvements in survival were observed after administration of G-CSF, findings attributed to SDF-1-dependent mobilization of cardiac stem cells. Our studies extend their observations through examination of the cardiovascular effects of DPP-4 inhibition in diabetic mice and by demonstrating that direct sitagliptin administration into the circulation of the ischemic mouse heart is not directly cardioprotective ex vivo, suggesting that acute reduction of cardiac DPP-4 activity is not sufficient to produce cardioprotection.

Our findings demonstrating that both sitagliptin and the GLP-1R agonist liraglutide upregulated levels of cardioprotective proteins in the nonischemic myocardium suggest a possible role for GLP-1 in the context of enhanced survival after DPP-4 inhibition and experimental MI. Nevertheless, we did not observe a sustained induction of cardioprotective proteins in sitagliptin-treated murine hearts when the same proteins were examined after MI. Moreover, we did not detect significant changes in infarct size or cardiac function after MI that might directly account for the improved survival seen with genetic or chemical reduction of DPP-4 activity. Hence, the precise mechanisms mediating the improvements in survival observed after pharmacological treatment with sitagliptin in diabetic mice or genetic reduction of DPP-4 activity in normoglycemic Dpp4−/− mice require further investigation, ideally through examination of whether DPP-4 inhibitors exert cardioprotective actions in Glp1r−/− mice.

Western blot analysis of proteins in nonischemic hearts demonstrated that both sitagliptin and metformin therapy induced an overlapping set of cardioprotective proteins. Metformin is thought to exert its cardioprotective actions through distinct mechanisms requiring activation of AMP kinase and endothelial nitric oxide (31). Intriguingly, administration of metformin has also been associated with reduction of DPP-4 activity (36) and increased circulating levels of GLP-1 in both rodent (37) and clinical studies (38), and we detected increased levels of GLP-1 in both metformin- and sitagliptin-treated mice. Accordingly, the extent to which therapy with sitagliptin and metformin produces an overlapping spectrum of actions reflecting similarities in their mechanism(s) of action through enhanced levels of GLP-1 requires further clarification.

Both metformin and sitagliptin significantly increased survival in diabetic mice, possibly due to a comparable reduction in blood glucose achieved with either agent. Hyperglycemia is a risk factor for a poor outcome after MI in humans (39), and there is considerable interest in determining whether intensive glucose control safely and consistently improves outcomes post-MI (40). Similarly, hyperglycemia is known to be associated with reduced survival and impaired LV function in mice after MI (41–43), and it seems likely that reduction in the severity of

TABLE 2

|                         | Control   | Sitagliptin | Metformin |
|-------------------------|-----------|-------------|-----------|
|                         | n = 6     | n = 6       | n = 5     |
| Aortic flow             |           |             |           |
| Heart rate (bpm)        | 441 ± 17  | 423 ± 19    | 526 ± 23* |
| VTImax (cm)             | 2.64 ± 0.14| 2.40 ± 0.08 | 2.39 ± 0.30|
| Aortic orifice diameter (mm) | 1.04 ± 0.04 | 1.06 ± 0.03 | 1.04 ± 0.03|
| LV stroke volume (µl)   | 22.38 ± 1.24| 21.10 ± 1.33| 20.34 ± 3.07|
| Cardiac output (ml/min) | 9.82 ± 0.46| 8.95 ± 0.71 | 10.70 ± 1.66|
| Mitral flow             |           |             |           |
| Heart rate (bpm)        | 447 ± 13  | 433 ± 16    | 792 ± 33  |
| Peak E velocity (cm/s)  | 67.6 ± 3.0| 62.6 ± 2.4  | 53.4 ± 6.2|
| Peak A velocity (cm/s)  | 48.0 ± 49 | 36.0 ± 40   | 54.6 ± 73 |
| Peak E/A ratio          | 1.47 ± 0.15| 1.89 ± 0.29 | 0.99 ± 0.08|
| LV chamber dimensions by M-mode |          |             |           |
| Heart rate (bpm)        | 493 ± 35  | 458 ± 15    | 541 ± 18  |
| LV end diastolic diameter (mm) | 4.28 ± 0.15| 4.28 ± 0.19 | 4.43 ± 0.16|
| LV end systolic diameter (mm) | 3.54 ± 0.27| 3.68 ± 0.21 | 3.89 ± 0.22|
| LV fractional shortening (%) | 17.3 ± 3.5 | 14.2 ± 1.2  | 12.4 ± 2.4 |

Data are means ± SE. The 12-week-old C57Bl/6 mice treated with control, sitagliptin, or metformin for 1 week before experimental cardiac ischemic injury were subjected to high-frequency ultrasound imaging. VTImax, velocity-time integral of Doppler flow waveform by tracing the maximal velocity. In mitral inflow, the peak E velocity represents the maximal velocity of the early diastolic wave caused by active left ventricular relaxation. The peak A velocity represents the maximal velocity caused by atrial contraction in late diastole. Acquisition of images and analysis of data were carried out as previously described (44,45). *P < 0.05 vs. control.
glucoregulation and provide a useful model for future studies. Given the increasing interest in using strategies based on DPP-4 inhibition for the treatment of diabetes, a more detailed understanding of the role of DPP-4 in the normal and diabetic cardiovascular system is clearly warranted.

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