Effects of dipeptidyl peptidase-4 inhibitor in insulin-resistant rats with myocardial infarction

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Abstract

Adverse cardiac remodeling after myocardial infarction (MI) leads to progressive heart failure. Obese-insulin resistance increases risks of MI and heart failure. Although dipeptidyl peptidase-4 (DPP4) inhibitor is known to exert cardioprotection, its effects on adverse remodeling after MI in obese-insulin-resistant rats are unclear. We hypothesized that DPP4 inhibitor reduces adverse left ventricular (LV) remodeling and LV dysfunction in obese-insulin-resistant rats with MI. Rats were fed either normal diet (ND) or high-fat diet (HFD) for 12 weeks to induce obese-insulin resistance, followed by left anterior descending coronary artery ligation to induce MI. Then, rats in each dietary group were divided into five subgroups to receive vehicle, enalapril (10 mg/kg/day), metformin (30 mg/kg/day), DPP4 inhibitor vildagliptin (3 mg/kg/day), or combined metformin and vildagliptin for 8 weeks. Heart rate variability (HRV), LV function, pathological and biochemical studies for LV remodeling, and cardiomyocyte apoptosis were determined. Obese-insulin-resistant rats had severe insulin resistance and LV dysfunction. HFD rats had a higher mortality rate than ND rats, and all treatments reduced the mortality rate in obese-insulin-resistant rats. Although all drugs improved insulin resistance, HRV, LV function as well as reduced cardiac hypertrophy and fibrosis, vildagliptin effectively reduced cardiomyocyte cross-sectional areas more than enalapril and was related to markedly decreased ERK1/2 phosphorylation. In ND rats with MI, metformin neither improved LV ejection fraction nor reduced cardiac fibrosis. The infarct size and transforming growth factor- β expression were not different among groups. In obese-insulin-resistant rats with chronic MI, DPP4 inhibitor vildagliptin exerts better cardioprotection than enalapril in attenuating adverse LV remodeling.

Key Words

- ▶ myocardial infarction
- ▶ vildagliptin
- ▶ metformin
- ▶ obese-insulin-resistant rats

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Introduction

Heart failure is a major health problem that can be found in over 23 million people worldwide (Bui *et al.* 2011). A common cause of heart failure is coronary artery disease. Following myocardial ischemia due to coronary artery occlusion, left ventricular (LV) remodeling is known to occur. Postmyocardial infarction (MI) remodeling has been divided into early-phase (within 72 h post MI) and late-phase LV remodeling (beyond 72 h) (Sutton & Sharpe 2000). During the late-phase LV remodeling, LV architecture is changed, including the distortion of LV shape, LV dilation, cardiomyocyte hypertrophy, scar formation, and increased wall stress, all of which could lead to LV dysfunction (Sutton & Sharpe 2000).

It has been shown that long-term high-fat diet (HFD) consumption could induce obese-insulin resistance (Apaijai *et al.* 2012) and is related to an increased risk of MI (Oh *et al.* 2005), promotion of arrhythmic death, and worsening of LV dysfunction in a myocardial ischemia/reperfusion (I/R) injury model (Apaijai *et al.* 2014, Liu *et al.* 2014). However, the roles of obese-insulin resistance in LV remodeling after MI are unclear. Furthermore, previous studies have shown that several oral antidiabetic drugs, including metformin and vildagliptin, exert cardioprotective effects against MI as well as heart failure in various animal models (Gundewar *et al.* 2009, Apaijai *et al.* 2014, Chinda *et al.* 2014).

Vildagliptin (a dipeptidyl peptidase-4 inhibitor) is an oral antidiabetic drug that could reduce hyperglycemia in type 2 diabetes (Mari *et al.* 2005) and improve insulin sensitivity and cardiac sympathovagal balance in obese-insulin-resistant rats (Apaijai *et al.* 2012, Pipatpipoon *et al.* 2013). In the heart, vildagliptin has been shown to exert beneficial effects in various models (Apaijai *et al.* 2013, Chinda *et al.* 2014, Miyoshi *et al.* 2014). It has been shown to preserve LV function and reduce the infarct size in cardiac I/R injury (Chinda *et al.* 2013, 2014, Apaijai *et al.* 2014). Moreover, vildagliptin reduced myocyte hypertrophy and perivascular and cardiac fibrosis in isoproterenol (Miyoshi *et al.* 2014), and transverse aortic constriction-induced heart failure (Takahashi *et al.* 2013). However, inconsistent findings exist as reported by Yin and colleagues who found that vildagliptin did not reverse LV remodeling in post-MI-induced heart failure rats (Yin *et al.* 2011). Moreover, the effects of vildagliptin, metformin, and combined vildagliptin and metformin on the heart with late-phase LV remodeling following MI in subjects with obese-insulin-resistant condition have not been investigated.

In this study, we investigated the effects of vildagliptin, metformin, and combined vildagliptin and metformin on cardiac sympathovagal balance, LV function, LV remodeling, including cardiomyocyte hypertrophy, cardiac fibrosis, and the molecular mechanisms responsible for these effects in rats with obese-insulin resistance. We hypothesized that combined vildagliptin and metformin exert superior cardioprotection against late-phase LV remodeling following MI in obese-insulin-resistant rats compared with monotherapy.

Materials and methods

All experiments in this study were approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (permit no. 28/2557), in compliance with NIH and ARRIVE guidelines. Male Wistar rats weighing 180–200 g were obtained from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Bangkok, Thailand. Rats were acclimatized for 7 days and housed in 12 h light:12 h darkness cycle with controlled temperature (25°C). Then, rats were divided into two dietary groups to receive either a normal diet (ND; a diet containing 19.8% energy from fat) or an HFD (a diet containing 59.3% energy from fat) for 12 weeks. At the 12th week, rats were subjected to permanent left anterior descending coronary artery (LAD) ligation to induce MI. Rats were allowed to acclimatize after surgery for 7 days when the inflammatory process of infarction was over and the scar formation was almost complete (Richer *et al.* 1999). After that, rats in each dietary group were subdivided into five subgroups ($n=6$ per group) to receive one of the following treatments: vehicle (V; 0.9% normal saline solution in an equal volume), DPP4 inhibitor vildagliptin (Vil; 3 mg/kg/day, LAF 237, Novartis), metformin (M; 30 mg/kg/day, Glucophage, Merck Serono), combined vildagliptin and metformin (C), and enalapril (E; 10 mg/kg/day, served as positive control, Berlin, Bangkok, Thailand). Rats were treated with one of these drugs for 8 weeks via gavage feeding. These doses were selected based on our previous studies; our previous study showed that 3 mg/kg/day of vildagliptin, 30 mg/kg/day of metformin, and 10 mg/kg/day of enalapril exerted cardioprotection in various models, including obese-insulin-resistant rats (Apaijai *et al.* 2013), an I/R injury model (Apaijai *et al.* 2014), and normal rats with chronic MI (Inthachai *et al.* 2015).

Chronic MI model

Rats were anesthetized using Zoletil (0.3 mL/kg, Virbac Laboratories, Carros, France) and Xylazine (0.075 mL/kg, Laboratorios Calier, S.A., Barcelona, Spain) via intramuscular injection. Surgery was performed under aseptic conditions, and thoracotomy was performed through the fourth intercostal space. The pericardium membrane was torn open, and the LAD was identified and ligated at 3 mm below its origin with 5-0 silk suture as previously described (Inthachai *et al.* 2015).

Determination of metabolic parameters

Body weight and food intake were recorded weekly. Blood samples were collected from the tail vein at baseline, week 12, and the end of treatment. Plasma glucose, total cholesterol, and triglyceride levels were determined using a commercial colorimetric assay kit (Erba Diagnostics Mannheim GmbH, Mannheim, Germany) (Apaijai *et al.* 2014). Plasma high-density lipoprotein (HDL) level was determined using a colorimetric assay kit (BioVision) (Apaijai *et al.* 2014). Plasma low-density lipoprotein (LDL) level was calculated using Friedewald equation (Apaijai *et al.* 2014). Plasma insulin levels were determined using a commercial ELISA kit (LINCO Research) (Pratchayasakul *et al.* 2011). The homeostatics model assessment (HOMA) index was used to assess insulin resistance, and the increased HOMA index indicates a higher degree of insulin resistance (Pratchayasakul *et al.* 2011). Plasma and cardiac 8-isoprostane levels were used as an oxidative stress marker and were determined using a commercial ELISA kit (OxiSelect, Cell Biolabs, Inc., San Diego, CA, USA) (Madian *et al.* 2011).

Heart rate variability determination

Heart rate variability (HRV) was used as an indicator of cardiac autonomic balance and was measured at baseline, week 4, week 8, week 12, and at the end of treatment. A lead II electrocardiogram was recorded for 20 min in conscious rats using PowerLab (ADInstruments, Sydney, Australia) with Chart 5.0 program (Apaijai *et al.* 2013). Power spectra of R–R interval variability were obtained using fast Fourier transform algorithm. A high-frequency band (HF; 0.6–3 Hz), a low-frequency band (LF; 0.2–0.6 Hz), and a very-low-frequency band (below 0.2 Hz) were analyzed using an analytical software program (Apaijai *et al.* 2013). Regarding the frequency domain analysis, the efferent vagal activity is a major contributor to the HF component,

and the LF component represents both sympathetic and parasympathetic activities (Chattipakorn *et al.* 2007). The increased LF/HF ratio is considered as an index of cardiac sympathovagal imbalance (Chattipakorn *et al.* 2007).

LV function determination

Echocardiographic study Echocardiographic study was performed at baseline, week 4, week 8, week 12, and the end of treatment. M-mode echocardiography was performed at the level of papillary muscles using Vivid-i (GE) under light anesthesia (2% isoflurane with 98% oxygen, Forane, Kent, UK). The percentage of fractional shortening (%FS) and the percentage of LV ejection fraction (%LVEF) were determined (Apaijai *et al.* 2014).

Pressure–volume loop study This procedure was performed at the end of treatment. Rats were anesthetized using a combination of Zoletil (50 mg/kg) and Xylazine (3 mg/kg) via intramuscular injection. The right carotid artery was identified and the pressure–volume catheter (Scisense, London, Ontario, Canada) was inserted and advanced into the LV. End-systolic pressure, end-diastolic pressure (EDP), maximum and minimum dP/dt , and stroke volume to body weight ratio (SV/BW) were determined using an analytical software program (LabScribe, Dover, NH, USA) as previously described (Apaijai *et al.* 2013).

Blood pressure determination Blood pressure was measured at the end of treatment. A volume–pressure recording cuff and occlusion cuff were attached to the tail. Blood pressure measurement was performed using a CODA2 channel noninvasive blood pressure system (Kent Scientific Corporation, Torrington, CT, USA). Blood pressure values, including systolic and diastolic pressures, were achieved by taking an average of 20 consecutive measurements at a steady state (Lekawanvijit *et al.* 2012).

Histological studies

Infarct size and fibrosis determination Mid-LV cross sections were stained with picosirius red and scanned (Aperio, Aperio Technologies Inc., San Diego, CA, USA) for infarct and fibrosis analysis. Infarct size was reported as the average of the proportions of endocardial and epicardial circumference by the infarct (Inthachai *et al.* 2015). Rats with nontransmural

infarction were excluded from the study. Furthermore, picrosirius red-stained collagen matrix deposition from the whole LV myocardium was selected for its intensity, and the proportional area was calculated using a preset algorithm for picrosirius red stain intensity (Inthachai *et al.* 2015).

Cardiomyocyte cross-sectional area determination Cardiomyocyte cross-sectional area was determined using hematoxylin and eosin (H&E) staining. Fifty LV cardiomyocytes with equal-sized nuclei were randomly selected for analysis of cross-sectional area from prescanned images. Cell surface areas were calculated by measuring the circumferential length of the myocyte using Aperio ScanScope (Aperio, Aperio Technologies Inc., San Diego, CA, USA) (Inthachai *et al.* 2015).

Western blot analysis The myocardial tissues for western blot study were obtained from the fresh heart at the end of the treatment period following chronic MI. Myocardial protein extracts were prepared by the homogenization of nitrogen-frozen myocardial tissues in a 300 μ L extraction buffer containing 20 mM Tris-HCl (pH 6.8), 1 mM sodium orthovanadate, 5 mM sodium fluoride, and a protease inhibitor. Total protein concentrations were determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories). Sixty micrograms of total protein were mixed with the loading buffer (5% betamercaptoethanol, 0.05% bromophenol blue, 75 mM Tris-HCl (pH 6.8), 2% SDS, and 10% glycerol) and loaded onto 10% SDS-acrylamide gels. Proteins were transferred to nitrocellulose membranes in a glycine/methanol-transferred buffer in a wet/tank blotting system (Bio-Rad Laboratories). Membranes were blocked in 5% skim milk in Tris-buffered saline and Tween buffer. Membranes were exposed to mouse polyclonal anti-rat BCL2, total connexin 43, p-connexin 43^{ser368}, total p38 mitogen-activated protein kinase (MAPK), p-p38 MAPK^{Thr180/Tyr182}, total NF- κ B, p-NF- κ B^{ser536}, transforming growth factor (TGF)- β (1:1000 dilution, Cell Signaling Technology), BAX, total ERK1/2, p-ERK1/2^{Thr202/Tyr204}, and β -actin (1:1000 dilution, Santa Cruz Biotechnology). Bound antibodies were detected using horseradish peroxidase conjugated with anti-rabbit IgG (1:2000 dilution, Cell Signaling Technology). The membranes were developed using the Clarity ECL Western Blotting Substrate (Bio-Rad) and densitometric analyses were carried out (Apaijai *et al.* 2014, Chinda *et al.* 2014).

Statistical analysis

Data were expressed as mean $\pm$ S.E.M. One-way ANOVA followed by Tukey's *post hoc* test was used to determine the differences among groups. Time-course values of echocardiographic parameters and HRV were determined using two-way repeated measures ANOVA. Mortality rate was determined using χ^2 -test. A *P*-value of <0.05 was considered as statistically significance.

Results

Effects of enalapril, vildagliptin, metformin, and combined drugs on metabolic parameters

At baseline (week 0 before HFD consumption), there were no differences in body weight, food intake, plasma glucose, insulin, total cholesterol, LDL, HDL, triglyceride levels, and HOMA index between the ND- and HFD-fed rats (Table 1). After 12-week HFD consumption, data showed that body weight, plasma insulin, total cholesterol, LDL levels, and HOMA index were increased in HFD-fed rats. However, there were no differences in food intake, plasma triglyceride, and HDL levels between the ND- and HFD-fed rats. Our data suggest that rats developed obese-insulin resistance after 12-week HFD consumption (Table 1).

Seven days after MI, rats received their assigned drugs for 8 weeks. At the end of treatment, we found that body weight was not changed in the ND and HFD groups, whereas LV/heart weight (HW) was increased in HFD-fed rats treated with vehicle (HFDV). All treatments reduced the LV/HW ratio in both MI rats; however, metformin did not reduce the LV/HW ratio in ND-fed rats (Table 2). Furthermore, we found that plasma glucose, insulin, and total cholesterol levels, and HOMA index were increased in HFDV compared with ND-fed rats treated with vehicle (NDV). Vildagliptin, metformin, and combined drugs reduced the levels of these parameters (Table 2).

Effects of enalapril, vildagliptin, metformin, and combined drugs on oxidative stress levels

We found that plasma and cardiac 8-isoprostane levels were higher in HFDV, compared with NDV. In ND-fed rats, treatment with enalapril, vildagliptin, and the combined drugs, but not metformin, reduced cardiac 8-isoprostane levels, whereas plasma 8-isoprostane level was not different among groups. In HFD-fed

Table 1 Effects of HFD consumption on metabolic parameters at baseline and 12 weeks after HFD consumption.

Metabolic parameters	Baseline		Week 12	
	ND	HFD	ND	HFD
Body weight (g)	178±5	181±3	446±12	533±10*
Food intake (g/day)	15±2	18±2	19±2	21±2
Plasma glucose (mg/dL)	128±4	138±9	122±10	130±5
Plasma insulin (ng/dL)	2.4±0.4	2.3±0.5	2.6±0.3	4.2±0.3*
HOMA index	13±0.6	14±0.1	14±0.7	24±0.2*
Plasma total cholesterol (mg/dL)	54±6	54±4	53±6	74±3*
Plasma triglyceride (mg/dL)	123±5	118±4	119±1	118±2
Plasma HDL (mg/dL)	20±5	23±6	24±4	22±3
Plasma LDL (mg/dL)	9±6	8±8	7±2	28±2*

ND, normal diet-fed rats; HFD, high-fat diet-fed rats; HOMA, homeostatic model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
* $P < 0.05$ vs ND.

rats, all treatments could reduce plasma and cardiac 8-isoprostane levels (Table 2).

Effects of enalapril, vildagliptin, metformin, and combined drug on HRV

At baseline, there was no difference in the LF/HF ratio between ND- and HFD-fed rats. The LF/HF ratio was increased after 8 weeks of HFD consumption, indicating the development of cardiac sympathovagal imbalance (Fig. 1A). After MI, HFDV rats had higher LF/HF ratio than NDV rats (Fig. 1B). In ND-fed rats, enalapril, vildagliptin, and combined drugs, but not metformin, reduced the LF/HF ratio, whereas all treatments could reduce the LF/HF ratio in HFD-fed rats (Fig. 1B).

Effects of enalapril, vildagliptin, metformin, and combined drug on LV function

Echocardiographic parameters At baseline, there were no differences in the %FS and %LVEF between ND- and HFD-fed rats. We found that %FS and %LVEF were decreased after 12-week HFD consumption (Fig. 2A and B). Furthermore, after treatment, HFDV demonstrated a worsened %LVEF compared with NDV, whereas the %FS did not differ between HFDV and NDV rats (Fig. 2C and D). All treatments improved %FS and %LVEF in HFD-fed rats. However, metformin did not improve %FS and %LVEF in ND-fed rats compared with NDV rats (Fig. 2C and D).

Hemodynamic parameters At the end of treatment, only enalapril could reduce the systolic pressure in ND- and HFD-fed rats compared with NDV and HFDV rats (Table 3). Moreover, our data showed that HFDV rats had higher

heart rate, higher EDP, and lower $dP/dt_{\min}$ compared with NDV rats. All treatments reduced EDP, and treatment with vildagliptin and combined drug increased $dP/dt_{\max}$ and $dP/dt_{\min}$ in HFD-fed rats. In ND-fed rats, treatment with vildagliptin and combined drug reduced EDP and increased $dP/dt_{\min}$, and enalapril could reduce only EDP. Metformin did not improve any hemodynamic parameters in ND-fed rats (Table 3). Our data indicated that HFDV rats had worsened LV dysfunction than NDV rats, and all treatments improved LV function after MI in obese-insulin-resistant rats, whereas metformin did not improve any LV function parameters in ND-fed rats (Table 3).

Effects of enalapril, vildagliptin, metformin, and combined drug on mortality rate and LV remodeling

For the perioperative mortality in the rats undergoing MI, the mortality rate was 27% in normal rats and 48% in obese-insulin-resistant rats during surgery. During 8 weeks of treatment period, we found that HFDV rats had a higher mortality rate after MI, and all treatments could reduce the mortality rate in HFD-fed rats (Fig. 3A). Our data also showed that connexin 43 (CX43) phosphorylation was lower in HFDV rats, and all treatment increased CX43 phosphorylation (Fig. 3B).

Infarct size, cardiac fibrosis, and cardiomyocyte cross-sectional area

After MI, the infarct size did not differ between groups (Fig. 3C). We also determined an apoptotic (BAX) and antiapoptotic protein (BCL2) ratio, and found that BAX/BCL2 ratio was not different among groups (Fig. 3D).

In this study, we determined cardiomyocyte cross-sectional areas from H&E stained heart tissues, and

Table 2 Effects of enalapril, vildagliptin, metformin, and combination of vildagliptin and metformin on metabolic parameters in ND- and HFD-fed rats with chronic MI.

Parameters	NDV	NDE	NDVil	NDM	NDC	HFDV	HFDE	HFDVil	HFDM	HFDC
Body weight (g)	545±3	525±5	475±8	490±6	520±4	635±9*	620±5*	655±4*	610±8*	640±9*
LV/HW	1.1±0.1	0.7±0.2*	0.8±0.1*	1.1±0.1	0.8±0.1*	1.2±0.1*	0.9±0.1†	0.8±0.1†	0.9±0.1†	0.8±0.1†
Visceral fat weight (g)	35±4	41±5	42±5	44±7	36±3	68±4*	62±5*	66±3*	65±9*	68±7*
Food intake (g/day)	17±1	16±1	17±1	19±1	15±1	17±1	14±1	14±1	15±1	16±1
Plasma glucose (mg/dL)	130±4	129±1	123±5	128±5	125±5	167±5*	155±7	142±5†	145±5†	143±6†
Plasma insulin (ng/dL)	3.0±0.2	3.0±0.7	2.8±0.4	3.1±0.4	3.1±0.4	4.3±0.1*	3.9±0.2	3.1±0.3†	2.7±0.2†	3.3±0.3†
HOMA index	17±0.5	17±0.2	15±0.7	18±0.7	17±0.7	32±0.9*	29±1	22±0.7†	20±0.6†	20±0.8†
Plasma total cholesterol (mg/dL)	60±9	65±6	68±3	68±5	61±3	151±13*	115±6†	117±6†	114±3†	113±2†
Plasma triglyceride (mg/dL)	120±5	121±9	135±6	118±7	133±10	123±10	124±10	119±5	122±6	129±5
Plasma HDL (mg/dL)	24±2	28±1	27±1	28±2	28±2	24±1	25±2	26±2	25±3	24±1
Plasma LDL (mg/dL)	12±4	13±6	14±2	16±3	16±3	102±5*	65±3†	67±2†	64±3†	63±3†
Plasma 8-isoprostane (ng/mL)	2.8±0.2	2±0.2	2.1±0.1	2.7±0.2	2.0±0.1	3.4±0.3*	2.3±0.1†	2.2±0.2†	2.5±0.1†	2.3±0.1†
Cardiac 8-isoprostane (ng/mg)	3.4±0.1	2.9±0.1*	2.7±0.1*	3.4±0.3	2.4±0.1*	3.9±0.1*	3.1±0.2†	3.1±0.2†	3.1±0.1†	2.9±0.1†

LV/HW, left-ventricular weight/heart weight; HOMA, homeostatic model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NDV, normal diet rats treated with vehicle; NDE, normal diet rats treated with enalapril; NDVil, normal diet rats treated with vildagliptin; NDM, normal diet rats treated with metformin; NDC, normal diet rats treated with combined drugs; HFDV, high-fat-fed rats treated with vehicle; HFDE, high-fat-fed rats treated with enalapril; HFDVil, high-fat-fed rats treated with vildagliptin; HFDM, high-fat-fed rats treated with metformin; HFDC, high-fat-fed rats treated with combined drugs.

* $P < 0.05$ vs NDV, † $P < 0.05$ vs HFDV.

cardiac fibrosis from picosirius-stained heart tissues (Fig. 4A, B, C and D). Our results showed that cardiac fibrosis and cardiomyocyte cross-sectional areas were increased in HFDV rats, and all treatments reduced cardiomyocyte cross-sectional areas in HFD-fed rats (Fig. 4A, B, C and D). However, only metformin did not reduce cardiomyocyte cross-sectional areas in ND-fed rats. Interestingly, vildagliptin and combined drug treatment markedly reduced cardiomyocyte cross-sectional areas compared with enalapril in both ND- and HFD-fed rats (Fig. 4B). Furthermore, all treatments decreased cardiac fibrosis in HFD-fed rats, whereas metformin did not reduce cardiac fibrosis in ND-fed rats (Fig. 4A, B, C and D).

Our results showed that all treatments could reduce p38 phosphorylation in HFD-fed rats (Fig. 5A). In ND-fed rats, only metformin did not reduce p38 phosphorylation (Fig. 5A). ERK1/2 phosphorylation tended to increase in HFDV rats, and only vildagliptin reduced ERK1/2 phosphorylation in both ND- and HFD-fed rats (Fig. 5B).

Furthermore, we found that all treatments decreased NF- κ B phosphorylation in HFD-fed rats but not in ND-fed rats (Fig. 5C). TGF- β expression was not different among groups (Fig. 5D).

Discussion

The major findings of this study are as follows: vildagliptin, metformin, and combined metformin and vildagliptin effectively attenuated the insulin-resistant condition in obese-insulin-resistant rats. Furthermore, vildagliptin, metformin, combined drugs, and enalapril reduced plasma and cardiac oxidative stress, improved HRV, and attenuated LV dysfunction and LV remodeling in obese-insulin-resistant rats. However, vildagliptin reduced cardiac hypertrophy better than other regimens in this model. In ND-fed rats, both vildagliptin and enalapril exerted cardioprotection against adverse LV remodeling. However, metformin exerted no benefit on late-phase LV remodeling after MI in ND-fed rats.

Figure 1

Effects of enalapril, vildagliptin, metformin, and combined drugs on HRV in normal and obese-insulin-resistant rats after chronic MI. The LF/HF ratio was increased after 8 weeks of HFD consumption. (A) $*P < 0.05$ vs ND. Enalapril, vildagliptin, metformin, and combined vildagliptin and metformin improved HRV in obese-insulin-resistant rats. In ND rats, enalapril, vildagliptin, and combined vildagliptin and metformin but not metformin reduced the LF/HF ratio. (B) $*P < 0.05$ vs NDV, $†P < 0.05$ vs HFDV. ND, normal diet-fed rats; HFD, high-fat diet fed rats; LF, low-frequency; HF, high-frequency; NDV, normal diet rats treated with vehicle; NDE, normal diet rats treated with enalapril; NDVil, normal diet rats treated with vildagliptin; NDM, normal diet rats treated with metformin; NDC, normal diet rats treated with combined drugs; HFDV, high-fat-fed rats treated with vehicle; HFDE, high-fat-fed rats treated with enalapril; HFDVil, high-fat-fed rats treated with vildagliptin; HFDM, high-fat-fed rats treated with metformin; HFDC, high-fat-fed rats treated with combined drugs.

After chronic MI, obese-insulin-resistant rats exhibited severe insulin resistance by increased HOMA index and plasma glucose level. Our data demonstrated that vildagliptin, metformin, and combined drugs improved insulin resistance without hypoglycemia. This finding is consistent with previous studies, in which these drugs improved insulin resistance in patients with type 2 diabetes mellitus (T2DM) and in animal models of insulin resistance without MI (Bailey 2005, Apaijai *et al.* 2013, Pintana *et al.* 2013, Derosa *et al.* 2014). Furthermore, our data suggested that vildagliptin and metformin also effectively improved metabolic parameters in an

animal model of obese-insulin resistance with chronic MI (Fig. 6).

In this study, only enalapril, an angiotensin-converting enzyme (ACE) inhibitor reduced systolic blood pressure and associated with reduced LDL cholesterol levels in obese-insulin-resistant rats. Clinical trials reported that enalapril reduced blood pressure by reduced angiotensin II level (Ocaranza *et al.* 2006) and LDL cholesterol level (Rachmani *et al.* 2000). Furthermore, the lower level of angiotensin II in addition to lower LDL cholesterol level could induce vasodilation (Kiliszek *et al.* 2007) and reduce systolic blood pressure. However, vildagliptin, metformin, and combined metformin and vildagliptin also reduced LDL cholesterol level without any changes in blood pressure. This finding consistent with previous reports in the heart failure model in which vildagliptin did not change blood pressure in rats with cardiac hypertrophy (Miyoshi *et al.* 2014), and metformin did not affect blood pressure and flow-mediated vasodilation in insulin-resistant patients with chronic HF (Wong *et al.* 2012). These data suggest that a reduction of LDL cholesterol level along with lower angiotensin II is necessary to reduce blood pressure in obese-insulin-resistant rats with chronic MI.

HRV is an indicator of cardiac sympathovagal balance as well as a predictor of sudden cardiac death after MI (Zuanetti *et al.* 1996, Chattipakorn *et al.* 2007), and depressed HRV is commonly found in MI patients (Chattipakorn *et al.* 2007). In our study, a depressed HRV was worsening in obese-insulin-resistant rats with chronic MI than in ND rats. Vildagliptin, metformin, combined vildagliptin and metformin, and enalapril improved HRV in obese-insulin-resistant rats. Vildagliptin, combined drugs, and enalapril, but not metformin, also improved HRV in ND rats. This finding was correlated with the reduction of oxidative stress level. Our data were consistent with several studies which reported that an increased oxidative stress level has a positive correlation with a degree of cardiac sympathovagal imbalance in insulin-resistant patients and in animal models (Manzella *et al.* 2001, Pavithran *et al.* 2008, Apaijai *et al.* 2013). This could be the explanation why vildagliptin and enalapril, but not metformin, improved HRV in ND-fed rats with MI because metformin did not reduce oxidative stress in this group (Fig. 6).

LV remodeling, including LV hypertrophy and cardiac fibrosis, is a common phenomenon that occurs following MI (Sutton & Sharpe 2000). In this study, vildagliptin, metformin, combined drugs, and enalapril reduced LV remodeling in obese-insulin-resistant rats. It is interesting that vildagliptin exerted better effect on LV remodeling because it could reduce cardiomyocyte hypertrophy

Figure 2

Effects of enalapril, vildagliptin, metformin, and combined drugs on %FS and %LVEF in normal and obese-insulin-resistant rats after chronic MI. %FS and %LVEF were decreased after 12-week of HFD consumption (A and B), $*P < 0.05$ vs ND. Enalapril, vildagliptin, metformin, and combined vildagliptin and metformin increased %FS and %LVEF in obese-insulin-resistant rats. In ND rats, enalapril, vildagliptin, and combined vildagliptin and metformin but not metformin increased %FS and %LVEF (C and D), $*P < 0.05$ vs NDV, $^+P < 0.05$ vs HFDV. ND, normal diet-fed rats; HFD, high-fat-diet fed rats; NDV, normal diet rats treated with vehicle; NDE, normal diet rats treated with enalapril; NDVII, normal diet rats treated with vildagliptin; NDM, normal diet rats treated with metformin; NDC, normal diet rats treated with combined drugs; HFDV, high-fat-fed rats treated with vehicle; HFDE, high-fat-fed rats treated with enalapril; HFDVII, high-fat-fed rats treated with vildagliptin; HFDM, high-fat-fed rats treated with metformin; HFDC, high-fat-fed rats treated with combined drugs.

greater than other regimens. Unlike our findings in obese-insulin-resistant rats, a previous study in normal rats with MI reported otherwise. Yin and colleagues reported that vildagliptin reduced neither cardiomyocyte size nor LV dysfunction when given at 3 weeks after MI (Yin *et al.* 2011), whereas our data suggested that vildagliptin exerted cardioprotection when given 7 days after MI in both normal and obese-insulin-resistant rats. This disparity in results could be influenced by the timing of drug administration and the models used in those studies. In this study, the mechanism responsible for the reduction of cardiomyocyte hypertrophy by vildagliptin was related to decrease ERK and p38 MAPK phosphorylation. ERK and p38 MAPK are the members of MAPK, which are activated by oxidative stress, and both ERK and p38 MAPK also regulate cardiomyocyte hypertrophy during LV remodeling. Yeh and colleagues reported that ERK phosphorylation was increased in mice after 4-week post MI and related with cardiac hypertrophy (Yeh *et al.* 2010). Vildagliptin and combined vildagliptin and metformin reduced ERK phosphorylation, thus attenuating cardiomyocyte hypertrophy in our rat model of obese-insulin-resistant and normal rats with chronic MI (Fig. 6).

Cardiac fibrosis has been identified as the developing process of heart failure after MI (Wang *et al.* 2012) and has been involved in p38 MAPK activation (Clerk & Sugden 2006). Our data demonstrated that all pharmacological interventions reduced p38 MAPK phosphorylation, whereas TGF- β , a fibrosis inducer, was unchanged. This finding was supported by a previous study which reported

that p38 activation could mediate renal fibrosis in a TGF- β independent manner (Stambe *et al.* 2004). Therefore, vildagliptin, metformin, and enalapril given during late-phase LV remodeling in this study could reduce cardiac fibrosis through p38 MAPK signaling in a TGF- β independent manner. In addition, NF- κ B, a transcription factor that regulates stress and inflammatory response, has been shown to be involved in LV remodeling process (Hamid *et al.* 2011). It has been shown that an activation of NF- κ B is related with inflammation and postinfarcted LV remodeling after MI (Hamid *et al.* 2011). In our study, all treatments reduced NF- κ B activity, suggesting that these drugs reduced inflammation and led to reduce LV remodeling in obese-insulin-resistant rats with chronic MI (Fig. 6).

Growing evidence demonstrates that LV dysfunction is markedly increased in late-phase LV remodeling (Pfeffer *et al.* 1991, Sutton & Sharpe 2000). Our results showed that cardiomyocyte hypertrophy and cardiac fibrosis and inflammation are the major contributors of LV dysfunction in our model. Vildagliptin, metformin, and enalapril reduced LV remodeling through several signaling pathways, including reduced ERK1/2, p38 MAPK, and NF- κ B. Moreover, these drugs also reduced oxidative stress and improved cardiac sympathovagal balance. These beneficial effects could be responsible for improved LV function in both ND-fed and obese-insulin-resistant rats with chronic MI (Fig. 6).

It has been shown that an increase of mortality rate after MI is associated with hyperglycemia and cardiac

Table 3 Effects of enalapril, vildagliptin, metformin, and combination of vildagliptin and metformin on blood pressure and LV function in ND- and HFD-fed rats with chronic MI.

Parameters	NDV	NDE	NDVII	NDM	NDC	HFDV	HFDE	HFDVII	HFDI	HFDC
SBP (mmHg)	145 ± 8	113 ± 4*	147 ± 2	144 ± 6	137 ± 3	154 ± 7	123 ± 3†	155 ± 3	153 ± 1	155 ± 1
DBP (mmHg)	105 ± 9	105 ± 1	102 ± 6	104 ± 7	111 ± 2	111 ± 7	106 ± 1	114 ± 1	119 ± 2	119 ± 2
HR (bpm)	251 ± 8	256 ± 5	277 ± 17	265 ± 13	245 ± 13	320 ± 17*	281 ± 10	287 ± 6	290 ± 4	297 ± 28
ESP (mmHg)	97 ± 4	101 ± 6	104 ± 7	99 ± 5	98 ± 3	79 ± 3	94 ± 2	95 ± 1	93 ± 3	101 ± 3
EDP (mmHg)	9 ± 2	5 ± 0.9*	4 ± 0.1*	9 ± 0.8	5 ± 0.7*	13 ± 0.8*	10 ± 0.2†	8 ± 0.6†	10 ± 0.6†	8 ± 0.7†
dP/dt _{max} (mmHg/s)	8763 ± 1304	9140 ± 2126	9321 ± 854	9342 ± 115	9361 ± 1591	6576 ± 1759	7633 ± 992	9478 ± 294†	7472 ± 382	10,131 ± 990†
dP/dt _{min} (mmHg/s)	4330 ± 486	4599 ± 444	5499 ± 272*	4203 ± 102	5615 ± 233*	3664 ± 487*	4286 ± 205	6615 ± 738†	3887 ± 244	6841 ± 687†
SV/BW (μL/g)	0.82 ± 0.14	0.84 ± 0.21	0.83 ± 0.21	0.83 ± 0.11	0.82 ± 0.20	0.79 ± 0.21	0.81 ± 0.22	0.80 ± 0.25	0.79 ± 0.18	0.81 ± 0.16

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; ESP, end-systolic pressure; EDP, end-diastolic blood pressure; SV/BW, stroke volume/body weight; NDV, normal diet rats treated with vehicle; NDE, normal diet rats treated with enalapril; NDVII, normal diet rats treated with vildagliptin; NDM, normal diet rats treated with metformin; NDC, normal diet rats treated with combined drugs; HFDV, high-fat-fed rats treated with vehicle; HFDE, high-fat-fed rats treated with enalapril; HFDVII, high-fat-fed rats treated with vildagliptin; HFDI, high-fat-fed rats treated with metformin; HFDC, high-fat-fed rats treated with combined drugs.

* $P < 0.05$ vs NDV, † $P < 0.05$ vs HFD.

sympathovagal imbalance (Chattipakorn *et al.* 2007, Luo *et al.* 2014). In this study, the mortality rate was significantly increased in obese-insulin-resistant rats. This finding is also consistent with our previous report in the rat model of ischemia-reperfusion injury (Apaijai *et al.* 2014). Fatal arrhythmia has been shown to commonly develop and could be responsible for increased mortality in post-MI (Goncalves 1995). CX43 is a gap junction protein that influenced the development of cardiac arrhythmias after MI (Roell *et al.* 2007). It has been shown that increased CX43 phosphorylation was associated with reduced cardiac arrhythmias (Roell *et al.* 2007). In this study, all pharmacological interventions reduced hyperglycemia, improved cardiac sympathovagal balance, and increased CX43 phosphorylation, thus leading to decreased mortality in this study (Fig. 6).

In our model, LV remodeling has occurred following MI, leading to LV dysfunction, and the remodeling has developed through these following pathways: (i) fibrosis, (ii) apoptosis, (iii) MAPK pathway that is responsible for cell growth and proliferation, (iv) oxidative stress, and (v) inflammation (Sutton & Sharpe 2000, Kwak *et al.* 2006, Hori & Nishida 2009). In this study, important markers of each pathway were determined to represent the effects of vildagliptin and metformin on LV remodeling such as (i) TGF- β as a fibrosis marker, (ii) BAX and BCL2 as apoptosis markers, (iii) p38 and ERK1/2 as markers of cell growth and proliferation, (iv) 8-isoprostane as an oxidative stress marker, and (v) NF- κ B as an inflammatory marker. Enalapril is known to reduce LV remodeling by directly inhibiting the ACE. However, the obese-insulin-resistant rats in this study had metabolic disturbances along with LV remodeling after chronic MI. In this study, we demonstrated that although enalapril, vildagliptin, and metformin effectively reduced LV remodeling via those pathways, only vildagliptin and metformin could attenuate the metabolic disturbances in these obese-insulin-resistant rats. These findings suggested that improvement of metabolic regulation could effectively attenuate adverse LV remodeling in obese-insulin-resistant rats with chronic MI.

Apart from the attenuation of insulin resistance, our data also demonstrated that vildagliptin exerted better cardioprotection than metformin through two pathways. First, vildagliptin exerted antioxidant effects in normal rats with chronic MI, whereas metformin could not. Second, vildagliptin reduced cell growth via a reduction of ERK1/2 phosphorylation in normal rats with chronic MI, whereas metformin could not. Therefore, our data suggest that vildagliptin exerted beneficial effects via a reduction

Figure 3

Effects of enalapril, vildagliptin, metformin, and combined drugs on mortality rate, CX43 phosphorylation, infarct size, and BAX/BCL2 ratio in normal and obese-insulin-resistant rats after chronic MI. Mortality rate was higher in HFDV, whereas enalapril, vildagliptin, metformin, and combined vildagliptin and metformin reduced mortality rate (A) and CX43 phosphorylation in obese-insulin-resistant rats after chronic MI (B). * $P < 0.05$ vs NDV, † $P < 0.05$ vs HFDV. Infarct size (C) and BAX/BCL2 ratio (D) were not different among groups. NDV, normal diet rats treated with vehicle; NDE, normal diet rats treated with enalapril; NDVII, normal diet rats treated with vildagliptin; NDM, normal diet rats treated with metformin; NDC, normal diet rats treated with combined drugs; HFDV, high-fat-fed rats treated with vehicle; HFDE, high-fat-fed rats treated with enalapril; HFDVII, high-fat-fed rats treated with vildagliptin; HFDM, high-fat-fed rats treated with metformin; HFDC, high-fat-fed rats treated with combined drugs.

Figure 4

Effects of enalapril, vildagliptin, metformin, and combined drugs on cardiomyocyte cross-sectional area and cardiac fibrosis in normal and obese-insulin-resistant rats after chronic MI. Representative picture of cardiomyocyte cross-sectional area from H&E and picosirius-stained heart tissue (A and C). Enalapril, vildagliptin, metformin, and combined vildagliptin and metformin decreased cardiomyocyte cross-sectional area in obese-insulin-resistant rats. In ND rats, enalapril, vildagliptin, and combined vildagliptin and metformin but not metformin decreased cardiomyocyte cross-sectional area. However, vildagliptin and combined drugs reduced cardiomyocytes cross-sectional area better than enalapril (B), * $P < 0.05$ vs their dietary control, † $P < 0.05$ vs enalapril-treated rats within group. Enalapril, vildagliptin, metformin, and combined vildagliptin and metformin reduced cardiac fibrosis in obese-insulin-resistant rats. In ND rats, enalapril, vildagliptin, and combined vildagliptin and metformin but not metformin reduced cardiac fibrosis (D), * $P < 0.05$ vs their dietary control. NDV, normal diet rats treated with vehicle; NDE, normal diet rats treated with enalapril; NDVII, normal diet rats treated with vildagliptin; NDM, normal diet rats treated with metformin; NDC, normal diet rats treated with combined drugs; HFDV, high-fat-fed rats treated with vehicle; HFDE, high-fat-fed rats treated with enalapril; HFDVII, high-fat-fed rats treated with vildagliptin; HFDM, high-fat-fed rats treated with metformin; HFDC, high-fat-fed rats treated with combined drugs.

Figure 5

Effects of enalapril, vildagliptin, metformin, and combined drugs on p38MAPK phosphorylation, ERK1/2 phosphorylation, NF-κB phosphorylation, and TGF-β expression in normal and obese-insulin-resistant rats after chronic MI. Vildagliptin and combined vildagliptin and metformin reduced ERK phosphorylation in ND and obese-insulin-resistant rats (A). Enalapril, vildagliptin, metformin, and combined vildagliptin and metformin reduced p38 MAPK phosphorylation in obese-insulin-resistant rats. In ND rats, enalapril, vildagliptin, and combined vildagliptin and metformin but not metformin decreased reduced p38 MAPK phosphorylation (B). Enalapril, vildagliptin, metformin, and combined vildagliptin and metformin reduced NF-κB phosphorylation in obese-insulin-resistant rats (C). TGF-β expression was not different among groups (D), * $P < 0.05$ vs NDV, † $P < 0.05$ vs HFDV. NDV, normal diet rats treated with vehicle; NDE, normal diet rats treated with enalapril; NDVII, normal diet rats treated with vildagliptin; NDM, normal diet rats treated with metformin; NDC, normal diet rats treated with combined drugs; HFDV, high-fat-fed rats treated with vehicle; HFDE, high-fat-fed rats treated with enalapril; HFDVII, high-fat-fed rats treated with vildagliptin; HFDM, high-fat-fed rats treated with metformin; HFDC, high-fat-fed rats treated with combined drugs.

in oxidative stress levels and ERK1/2 phosphorylation in normal rats with chronic MI.

Even though our data indicated that DPP4 inhibitor vildagliptin attenuated adverse LV remodeling in obese-insulin-resistant rats, the effects of DPP4 inhibitors on HF in the clinical setting are still unclear. At this time, there are five available commercial DPP4 inhibitors: alogliptin,

sitagliptin, vildagliptin, saxagliptin, and linagliptin. A report from the EXAMINE trial showed that alogliptin did not increase the risk of a heart failure outcome in T2DM patients (White *et al.* 2013). For vildagliptin and sitagliptin, the reports from clinical trials still have conflicting data. Some studies reported that sitagliptin and vildagliptin increased neither the risk of adverse

Figure 6

A diagram summarized the effects of vildagliptin, metformin, combined drugs, and enalapril in the obese-insulin-resistant rats with chronic MI. All treatment reduced insulin resistance, reduced oxidative stress, improved cardiac sympathovagal balance, reduced LV remodeling, and increased gap function protein function, leading to improved LV function and reduced mortality rate. CX43, connexin 43; LV, left ventricle. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-16-0096>.

cardiovascular events nor the rate of hospitalization for HF (McInnes *et al.* 2015, Wang *et al.* 2015, Fu *et al.* 2016). However, Suh and colleagues reported otherwise, as they showed that sitagliptin and vildagliptin increased the hospitalization rate for HF after 30 days of medication (Suh *et al.* 2015). For saxagliptin, the SAVOR-TIMI53 trial demonstrated that saxagliptin increased the rate of hospitalization for heart failure (Scirica *et al.* 2013). Because these drugs have different forms and different affinities to DPP4, the effect of these DPP4 inhibitors on HF could depend on the type of the drug used in that trial, and this point will need further investigation.

Unlike vildagliptin and enalapril, metformin exerted cardioprotection against MI only in obese-insulin-resistant rats. Previous clinical study reported that metformin did not improve LVEF in ST-elevated MI (STEMI) nondiabetic patients (Lexis *et al.* 2014a). However, metformin improved clinical outcomes in metabolic syndrome patients undergoing percutaneous coronary intervention (Li *et al.* 2014) and reduced MI size in diabetic patients with STEMI (Lexis *et al.* 2014b). Furthermore, metformin has been shown to improve cardiac function in normal rats with chronic MI (Soraya *et al.* 2012, 2014). All of these findings suggested that metformin exerts cardioprotection in obese-insulin-resistant rats but fails to protect the heart against MI in normal rats. Our findings are consistent with these reports because metformin did not attenuate cardiac autonomic imbalance, LV dysfunction, and LV remodeling in normal rats after MI, whereas it exhibited the beneficial effects in obese-insulin-resistant rats mainly by reducing insulin resistance and exerted cardioprotection after MI in obese-insulin-resistant rats. Data from this study indicated that oxidative stress influenced LV remodeling after MI; metformin did not exert direct antioxidative stress property because metformin did not exert cardioprotection against MI in normal rats. However, vildagliptin exhibited a direct antioxidative stress property in normal rats with MI.

Conclusions

Vildagliptin, metformin and enalapril attenuated insulin resistance and cardiac sympathovagal imbalance, improved LV function, decreased cardiac fibrosis, and reduced the mortality rate in obese-insulin-resistant rats with MI. However, vildagliptin exerted better efficacy in reducing cardiomyocytes hypertrophy than metformin and enalapril. Moreover, vildagliptin, but not metformin, also provided benefits in improving cardiac sympathovagal

balance, reducing cardiomyocyte hypertrophy and cardiac fibrosis, and improving LV function in nonobese-insulin-resistant rats with MI.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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