RESEARCH

Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury

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Abstract

Sodium-glucose cotransporter 2 inhibitor (SGLT2-i) effects on cardiac ischemia/reperfusion (I/R) injury are unclear. Unlike SGLT2-i, dipeptidyl peptidase 4 inhibitors (DPP4-i) have shown effective cardioprotection in cardiac I/R injury. We aimed to investigate whether SGLT2-i reduces myocardial dysfunction and myocardial injury to a greater extent than DPP4-i in obese insulin-resistant rats with/without cardiac I/R injury. The high-fat (HF) diet-induced obese insulin-resistant rats were divided into 4 groups and received the following treatments for 28 days: vehicle (HFV); vildagliptin at a dosage of 3 mg/kg/day (HFVil); dapagliflozin at a dosage of 1 mg/kg/day (HFDa) and combination drugs (HFDaVil). At the end, I/R injury was induced by a 30-min left anterior descending coronary occlusion and 120-min reperfusion. Dapagliflozin showed a greater efficacy than vildagliptin in improving the metabolic impairments, low frequency/high frequency (LF/HF) ratio, systolic blood pressure and left ventricular (LV) function in comparison to HFV rats. In cardiac I/R injury, dapagliflozin had a greater efficacy than vildagliptin in decreasing mitochondrial DRP1, cleaved caspase 3, LV dysfunction and infarct size in comparison to HFV rats. However, the combined therapy showed the greatest efficacy in attenuating LV dysfunction, mitochondrial DRP1 and infarct size in comparison to HFV rats. In conclusion, dapagliflozin has a more pronounced effect than vildagliptin in obese insulin-resistant rats for the improvement of LV function. In rats with cardiac I/R injury, although dapagliflozin had a greater efficacy on cardioprotection than vildagliptin, the combined therapy exerted the highest cardioprotective effects potentially by reducing mitochondrial fission.

Key Words

- ▶ SGLT2 inhibitors
- ▶ dapagliflozin
- ▶ DPP4 inhibitors
- ▶ vildagliptin
- ▶ cardioprotection
- ▶ pre-diabetes
- ▶ cardiac I/R injury

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Introduction

The sodium-glucose cotransporter 2 inhibitors (SGLT2-i) are a new class of anti-diabetic drugs, which exhibit a blood glucose-lowering effect by inhibiting glucose reabsorption at the proximal tubule of the nephron, resulting in decreased renal glucose reabsorption and increased renal

glucose excretion, thus improving glycemic control (Vrhovac *et al.* 2015). A recent clinical trial, EMPA-REG OUTCOME, demonstrated that SGLT2-i empagliflozin reduced cardiovascular morbidity and mortality in patients with type 2 diabetes (T2DM) who are at a high risk

of cardiovascular events. Although the benefits of SGLT2-i are potentially through cardiometabolic regulation, this effect of SGLT2-i in comparison with other anti-diabetic drugs, especially in pre-diabetic conditions both with and without myocardial ischemia/reperfusion (I/R) injury, as well as its underlying mechanism have never been investigated.

Incretin-based therapy using dipeptidyl peptidase 4 inhibitors (DPP4-i) is a well-known effective glycemic control remedy. The cardioprotective effects of DPP4-i beyond its glycemic regulating effects have been reported to lead to an improvement in cardiac function in high-fat diet (HFD)-induced pre-diabetes rat models (Apaijai *et al.* 2013, Tanajak *et al.* 2017). Treatment with DPP4-i provided effective cardioprotection in rats with myocardial I/R injury (Chinda *et al.* 2014). In healthy volunteers, treatment with vildagliptin at the highest daily therapeutic dose or a fourfold higher dose did not change the QT interval, nor did it have adverse effects on cardiac conduction (He *et al.* 2011). However, the benefit of its glycemic control as well as cardioprotection in obese insulin-resistant with cardiac I/R injury compared to SGLT2-i is not known.

This study aimed to investigate the effects of SGLT2-i (dapagliflozin), DPP4-i (vildagliptin) and a combined therapy of both agents on metabolic regulation, blood chemistry, blood pressure, heart rate variability (HRV) and LV function in HFD- induced pre-diabetic rats. Moreover, the therapeutic effects of these drugs on cardiac arrhythmia, myocardial infarction size and LV function, as well as the underlying mechanisms under cardiac I/R injury were investigated in HFD-induced obese insulin-resistant rats. Our hypothesis is that dapagliflozin provides more effective metabolic regulation and cardioprotection than vildagliptin in HFD-induced obese insulin-resistant rats, both with/without cardiac I/R injury and also that the combined therapy exerts superior cardioprotection in these rats, when compared with single regimens.

Materials and methods

Ethical approval

All experimental protocols in this study were approved by the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee (Permit No. 46/2558), in compliance with NIH guidelines and in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.* 2010).

Experimental animals

After 1 week of acclimatization, all rats were randomized into 2 groups and were given either ND ($n=12$) or HFD ($n=48$) for 12 weeks. At week 12, ND rats were fed continuously with ND and vehicle (NDV) for 28 days. At week 12, HFD rats were subdivided into 4 experimental groups ($n=12$ /group), and each group received one of the following treatments via oral gavage feeding for 28 days: (1) HFD rats received vehicle (HFV); (2) HFD rats received vildagliptin 3mg/kg/day (HFVil); (3) HFD rats received dapagliflozin 1mg/kg/day (HFDa) or (4) HFD rats received the combination of vildagliptin 3mg/kg/day and dapagliflozin 1mg/kg/day (HFDaVil). At the end of week 12 of the assigned diet treatment and again after 28 days of drug treatments, the metabolic parameters, blood chemistry, 24-h urine volume, urinary glucose excretion (UGE), 24-h UGE, blood pressure and HRV were investigated. Then, *in vivo* myocardial I/R were induced by left anterior descending (LAD) coronary artery ligation for 30min followed by reperfusion for 120min. During the myocardial I/R procedure, lead II ECG was used to record arrhythmia parameter measurements. LV function was determined using a pressure–volume (P–V) loop at the beginning of the I/R procedure (baseline) and throughout the cardiac I/R study. At the end of the cardiac I/R procedure, the heart was removed rapidly and then perfused with 20mL cold 0.9% NSS. Finally, the removed hearts were used to assess the levels of myocardial infarction, cardiac mitochondrial function and protein expression. The myocardium was divided into the remote area and ischemic area for measurements of cardiac mitochondrial function, cardiac lipid peroxidation, tissue proteins expression, mitochondrial protein expression and myocardial infarction area. The experimental design, the number of rats and the details of data sets for analysis are listed in Fig. 1.

Myocardial I/R surgical procedure

Rats were anesthetized by an intramuscular injection of Zoletil (zolazepam and tiletamine) 50mg/kg in combination with xylazine 3mg/kg and were ventilated via a tracheotomy tube by Harvard Rodent Ventilator Model 683 (Harvard Apparatus, Holliston, MA, USA) (Pongkan *et al.* 2016). Lead II ECG was monitored continuously throughout the entire I/R procedure. A left intercostal thoracotomy incision was performed, and then the LAD coronary artery was identified and ligated at approximately 2mm distal to the origin of the left coronary, by a 5-0 silk suture to induce myocardial infarction. The LAD was

Figure 1

Diagram of the experimental protocol, number of animals and data set for analysis of this study. HFD, high-fat diet; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with vildagliptin and dapagliflozin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; HPLC, high-performance liquid chromatography; MMP, mitochondrial membrane potential; ND, normal diet; NDV, normal diet treated with vehicle; ROS, reactive oxygen species; TEM, transmission electron microscopy; TTC, triphenyltetrazolium chloride.

ligated for 30 min, the procedure being followed by 120 min of reperfusion. Myocardial ischemia was confirmed by ST elevation on the ECG recording (Pongkan *et al.* 2016).

Pressure-volume (P-V) loop for LV function assessment during I/R

During myocardial I/R study, an admittance-based P-V catheter (Transonic Scisense, Ontario, Canada) was used to investigate LV function throughout the protocol. After the rats were anesthetized, the right common carotid artery (Rt. CCA) was identified. The P-V loop catheter was inserted into the Rt. CCA and advanced into the LV chamber for hemodynamic monitoring. The catheter was connected to the recording system operated by LabScribe 2 software (iWorx System, Dover, NH, USA) (Tanajak *et al.* 2016, 2017). LV function parameters including heart rate (HR), end-systolic pressure (ESP), end-diastolic pressure (EDP), maximum pressure ($P_{\max}$), minimum pressure ($P_{\min}$), $dP/dt_{\max}$, $dP/dt_{\min}$, stroke volume (SV), ejection fraction (%EF), end-systolic volume (ESV), end-diastolic volume (EDV) and stroke work (SW) were determined.

Determination of arrhythmia parameters during cardiac I/R procedure

The occurrence of cardiac arrhythmia was characterized using the Lambeth Conventions method (Curtis *et al.* 2013). Arrhythmia scores were determined using the criteria described in previous studies (Pongkan *et al.* 2015, 2016).

Infarct size measurement

At the end of the I/R experiment, the hearts were removed and irrigated with cold 0.9% NSS to wash out any residual blood from the chambers and great vessels. The LAD was re-occluded at the same site that was used previously during the ischemic period. The catheters were inserted into the aorta and retrogradely perfused with a 1% Evans blue dye infusion. The area that could not be infused with the dye was defined as the area of no blood flow during the ischemic period. Then, the heart was frozen and cut horizontally into 1-mm thick slices from the apex to 1 mm above the site of occlusion. The heart

Table 1 The effects of vildagliptin, dapagliflozin and combined therapy on blood pressure in HFD rats after treatment for 4 weeks prior myocardial I/R injury.

Parameters	Groups				
	NDV	HFV	HFVil	HFDa	HFDaVil
SBP (mmHg)	126 ± 4	147 ± 3*	145 ± 4*	118 ± 5 ^{†,‡}	124 ± 6 ^{†,‡}
DBP (mmHg)	93 ± 3	98 ± 2	94 ± 6	91 ± 5	98 ± 5
MAP (mmHg)	104 ± 4	126 ± 4*	121 ± 4*	105 ± 3 ^{†,‡}	105 ± 3 ^{†,‡}

Data are shown as mean ± S.E.M., $n = 10$ – 12 /group.

* $P < 0.05$ vs NDV; [†] $P < 0.05$ vs HFV; [‡] $P < 0.05$ vs HFVil.

DBP, diastolic blood pressure; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with dapagliflozin and vildagliptin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; MAP, mean arterial pressure; NDV, normal diet treated with vehicle; SBP, systolic blood pressure.

slices were incubated in 37°C, 1% triphenyltetrazolium chloride (TTC) for 15 min (Pongkan et al. 2016). After incubation, the area with viable tissue was red, whereas the area that did not stain with Evans blue was defined as the area at risk (AAR). The area that was neither blue nor red (white area) was defined as the infarct site (Riess et al. 2009, Chinda et al. 2014, Pongkan et al. 2015). The myocardial infarct size was measured using Image tools software, version 3.0 and was calculated, depending on the weight of each tissue slice in accordance with the formula by Riess et al. (Riess et al. 2009, Chinda et al. 2014, Pongkan et al. 2015). The levels of myocardial infarct size were presented as a percentage of myocardial infarct size per AAR.

Cardiac mitochondrial function and morphology measurements

After the cardiac I/R study, rats were killed, and the hearts were removed rapidly. The hearts were perfused with cold 0.9% NSS. Cardiac mitochondria were obtained from both remote and ischemic myocardium using a differential centrifugation technique as described previously (Thummasorn et al. 2011, Tanajak et al. 2017). Cardiac mitochondrial function, which is defined by the following parameters: cardiac mitochondrial reactive oxygen species (ROS), cardiac mitochondrial membrane potential change (red/green fluorescence intensity ratio) and cardiac mitochondrial swelling (absorbance intensity) were investigated. The details of the procedures for obtaining cardiac mitochondrial function and morphology measurements have been described previously (Tanajak et al. 2017). An increase in the fluorescent intensity indicates an increase in mitochondrial ROS production, which suggests increased

oxidative stress levels. A decrease in the red/green fluorescence intensity ratio indicates an increase in cardiac mitochondrial membrane depolarization, whereas a decrease in mitochondrial absorbance indicates cardiac mitochondrial swelling (Thummasorn et al. 2011, Tanajak et al. 2017). Moreover, cardiac mitochondrial morphology was also demonstrated at 15,000× using a JEM-2200FS field emission electron microscope to confirm changes in mitochondrial structure or swelling.

Plasma and cardiac lipid peroxidation assessments

Blood was collected from the descending aorta after the rat was killed at the end of the myocardial I/R protocol, and then plasma was obtained and used for the study of lipid peroxidation levels. Tissue samples were obtained from both the remote and ischemic myocardium from the same heart that was used in the cardiac mitochondrial study. The homogenized heart tissues and plasma malondialdehyde (MDA) concentrations were determined using a high-performance liquid chromatography (HPLC)-based assay (Thermo Scientific) (Apaijai et al. 2013, Tanajak et al. 2017). Plasma and cardiac MDA were mixed with H_3PO_4 and thiobarbituric acid (TBA) to produce TBA-reactive substances (TBARS). Plasma and cardiac TBARS concentrations were determined directly from a standard curve and reported as equivalent to the MDA concentration. Cardiac MDA levels are presented as normalized units by protein concentration (Apaijai et al. 2013, Tanajak et al. 2017).

Statistical analysis

Data are reported as mean ± S.E.M. in the tables and reported as medians and interquartile range in the graphs. Normal distribution of the data was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk normality test. Comparison between groups of the non-normally distributed data were performed using nonparametric statistical analysis (Kruskal–Wallis and Mann–Whitney) followed by a *post hoc* Bonferroni correction. Comparisons between groups for normally distributed data were performed using a one-way ANOVA followed by a *post hoc* Bonferroni correction. VT/VF incidence was compared among groups using a χ^2 test. Data were analyzed using SPSS statistics software, version 22. All statistical tests conducted were two tailed, and results were considered to be statistically significant when the P value was less than 0.05 ($P < 0.05$).

Results

Dapagliflozin provided better outcomes than vildagliptin in metabolic regulation, improved cardiac morphometric and urinary profiles in pre-diabetic rats

HFV rats had significantly decreased HDL-C levels and significantly increased body weight, visceral fat, heart weight, heart weight/body weight ratio, plasma insulin, HOMA index, AUCg, plasma TC, plasma LDL-C and plasma FGF21 levels, when compared with NDV rats

(Supplementary Table 1, see section on [supplementary data](#) given at the end of this article). Regarding plasma TG levels, there was not changed in all groups (Supplementary Table 1). After treatment, all vildagliptin-treated rats had a decrease in plasma insulin, HOMA index, AUCg and plasma FGF21 levels, when compared with the HFV rats. Treatments with dapagliflozin and combined therapy showed a greater efficacy than vildagliptin in decreasing body weight, visceral fat, heart weight, heart weight/body weight ratio, plasma insulin, plasma glucose, HOMA index

Table 2 The effects of vildagliptin, dapagliflozin and combined therapy on heart rate variability (HRV) of HFD rats after treatment for 4 weeks prior myocardial I/R injury.

Parameters	Groups				
	NDV	HFV	HFVil	HFDa	HFDaVil
Mean HR (bpm)	408 ± 18	497 ± 18*	459 ± 11*,†	417 ± 17†,‡	413 ± 17†,‡
SDNN (ms)	4.1 ± 0.5	3.9 ± 0.5	3.9 ± 0.5	4.4 ± 0.6	4.0 ± 0.7
rMSSD (ms)	2.1 ± 0.1	2.2 ± 0.1	2.3 ± 0.2	2.2 ± 0.2	2.1 ± 0.1
Total power (TP; ms ²)	420 ± 45	370 ± 58	490 ± 72	409 ± 87	476 ± 69
VLF (ms ²)	59 ± 7	64 ± 6	63 ± 6	63 ± 7	65 ± 7
LF (ms ²)	61 ± 5	71 ± 8	61 ± 13	50 ± 8	80 ± 13
HF (ms ²)	322 ± 37	300 ± 23	358 ± 47	308 ± 72	357 ± 45
LFnu	0.13 ± 0.02	0.28 ± 0.04*	0.23 ± 0.02*,†	0.16 ± 0.02†,‡	0.16 ± 0.01†,‡
HFnu	0.85 ± 0.03	0.57 ± 0.06*	0.72 ± 0.05*,†	0.87 ± 0.02†,‡	0.88 ± 0.02†,‡
LF/HF ratio	0.21 ± 0.03	0.48 ± 0.03*	0.34 ± 0.06*,†	0.23 ± 0.02†,‡	0.24 ± 0.02†,‡

Data are shown as mean ± S.E.M., *n* = 10–12/group.

**P* < 0.05 vs NDV; †*P* < 0.05 vs HFV; ‡*P* < 0.05 vs HFVil.

HF, high frequency; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with dapagliflozin and vildagliptin; HFnu, normalized HF (HF power to total power); HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; HR, heart rate; LF, low frequency; LFnu, normalized LF (LF power to total power); LF/HF, low frequency/high frequency; NDV, normal diet treated with vehicle; rMSSD, square root of the mean squared differences of successive NN-intervals; SDNN, standard deviation of all normal to normal intervals; TP, total power; VLF, very low frequency.

and AUCg. In addition, dapagliflozin-treated rats had significantly increased plasma FGF21 levels, urine glucose excretion (UGE), 24-h urine volume and 24-h UGE, when compared with NDV, HFV and HFVil rats

(Supplementary Table 1). However, all pharmacological interventions demonstrated a similar efficacy on the increase of HDL-C levels and the decrease of TC and LDL-C levels, when compared with the HFV group.

Figure 3

The effects of vildagliptin, dapagliflozin and combined therapy on cardiac arrhythmia and cardiac conductivity-related proteins in pre-diabetic rats with cardiac I/R injury. (A) Time to 1st VT/VF, *n* = 12 per group; (B) arrhythmia score, *n* = 12 per group; (C) number of VT/VF, *n* = 12 per group; (D) VT/VF incidence, *n* = 12 per group; (E) p-Cx43 Ser₃₆₈ expression in the ischemic area normalized with that in the remote area, *n* = 5–6 per group and (F) representative Western blot bands of p-Cx43 Ser₃₆₈ and Cx43 proteins expression in the ischemic area and the remote area. **P* < 0.05 vs NDV and †*P* < 0.05 vs HFV. Cx43, connexin 43; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with vildagliptin and dapagliflozin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; NDV, normal diet treated with vehicle; p-Cx43 Ser₃₆₈, phosphorylation of Cx43 at serine 368; VF, ventricular fibrillation; VT, ventricular tachycardia.

Dapagliflozin, but not vildagliptin, led to restoration of normal blood pressure in pre-diabetic rats

Blood pressure data are shown in Table 1. HFV rats had significantly increased systolic blood pressure (SBP) and mean arterial pressure (MAP), when compared with the NDV group. Dapagliflozin and combined therapy led to significantly reduced SBP and MAP, when compared with the HFV group, and the levels being restored to within-normal limits. However, this blood pressure-lowering effect was not observed in vildagliptin-treated rats.

Dapagliflozin exhibited a greater efficacy on LV function and HRV improvements than vildagliptin in pre-diabetic rats

LV systolic and diastolic functions were evaluated, and these data are shown in Fig. 2. The representative echocardiographic images in M-mode at 4 weeks after treatment are shown in Fig. 2A. HFV rats had a decreased %LVEF and had increased levels of LVIDs, LVPWs and LVPWd, when compared with NDV rats (Fig. 2B). The representative echocardiographic images

Figure 4

The effects of vildagliptin, dapagliflozin and combined therapy on myocardial infarction, myocardial lipid peroxidation and cardiac apoptotic protein expression in pre-diabetic rats with cardiac I/R injury. (A) Representative heart section for myocardial infarction, $n=6$ per group; (B) area at risk, $n=6$ per group; (C) myocardial infarct size, $n=6$ per group; (D) cardiac MDA at ischemic area, $n=5-6$ per group; (E) cardiac MDA at remote area, $n=5-6$ per group; (F) Bax/Bcl-2 ratio expression in the ischemic area normalized with that in the remote area, $n=5-6$ per group; (G) caspase 3 in the ischemic area normalized with that in the remote area, $n=6$ per group; (H) cleaved caspase 3 in the ischemic area normalized with that in the remote area, $n=6$ per group; and (I) representative Western blot bands of Bax, Bcl-2, cleaved caspase 3 and caspase 3 proteins expression in the ischemic area and the remote area. * $P<0.05$ vs NDV; † $P<0.05$ vs HFV; ‡ $P<0.05$ vs HFVil; and # $P<0.05$ vs HFDa. Bax, Bcl2-associated X protein; Bcl-2, B-cell lymphoma 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with vildagliptin and dapagliflozin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; MDA, malondialdehyde; NDV, normal diet treated with vehicle.

Table 3 The effects of vildagliptin, dapagliflozin and combined therapy on cardiac function during the myocardial I/R injury protocol.

Parameters	NDV			HFV		
	Baseline	Ischemia	Reperfusion	Baseline	Ischemia	Reperfusion
HR (bpm)	226 ± 16	338 ± 22*	355 ± 26*	316 ± 12 [‡]	417 ± 31*	399 ± 22*
ESP (mmHg)	123 ± 4	77 ± 5*	95 ± 6*, [†]	94 ± 8 [‡]	56 ± 5*	65 ± 5*
EDP (mmHg)	4 ± 1	11 ± 3*	5 ± 2 [†]	9 ± 2 [‡]	15 ± 4*	14 ± 3*
P _{max} (mmHg)	136 ± 8	122 ± 17	122 ± 9	134 ± 7	131 ± 17	136 ± 15
P _{min} (mmHg)	5 ± 2	5 ± 3	6 ± 2	6 ± 3	6 ± 2	7 ± 2
dP/dt _{max} (mmHg/s × 10 ³)	7.81 ± 0.41	4.47 ± 0.34*	6.22 ± 0.50*, [†]	5.05 ± 0.31 [‡]	3.72 ± 0.3*	4.05 ± 0.49*
dP/dt _{min} (mmHg/s × 10 ³)	6.51 ± 0.38	4.70 ± 0.23*	6.01 ± 0.20 [†]	5.04 ± 0.26 [‡]	4.15 ± 0.24*	3.96 ± 0.26*
ESV (μL)	3 ± 1	13 ± 2*	8 ± 3*, [†]	9 ± 2 [‡]	20 ± 4*	19 ± 4*
EDV (μL)	294 ± 18	337 ± 11*	298 ± 31 [†]	329 ± 12 [‡]	368 ± 28*	375 ± 16*
SV (μL)	303 ± 21	192 ± 19*	265 ± 11 [†]	238 ± 18 [‡]	154 ± 10*	169 ± 24*
EF (%)	80 ± 2	67 ± 4*	63 ± 4 [†]	64 ± 3 [‡]	52 ± 3*	49 ± 3*
Stroke work (mmHg × μL × 10 ³)	51.8 ± 2.8	33.0 ± 1.8*	46.6 ± 3.1 [†]	38.9 ± 3.1 [‡]	28.8 ± 1.5*	29.5 ± 2.6*

in pulsed-wave Doppler mode demonstrating the cardiac performance, at 4 weeks after treatment, are shown in Fig. 2C. Moreover, decreased early/atrial (late) (E/A) ratio, increased deceleration time (DT) and increased isovolumetric ventricular relaxation time (IVRT) were observed in HFV rats, compared with NDV rats (Fig. 2D).

HFVil rats had significantly increased %LVEF and decreased LVIDs, when compared with HFV rats (Fig. 2B), whereas HFDA and HFDAVil rats had increased %LVEF with decreased LVIDs, LVPWs and LVPWD, when compared with HFV and HFVil rats (Fig. 2B). All therapeutic groups showed a similar outcome in cardiac diastolic improvement, identified by an increased E/A ratio, decreased DT and decreased IVRT, when compared with HFV rats (Fig. 2D).

HFV rats had a significantly increased mean HR, normalized LF (LF power to total power; LFnu), LF/HF ratio and decreased normalized HF (HF power to total power; HFnu), when compared with NDV rats (Table 2). Treatment with vildagliptin significantly decreased mean HR, LFnu, LF/HF ratio and increased HFnu, when compared with HFV rats. However, dapagliflozin and the combined therapy had a greater efficacy than vildagliptin in leading to a decreased mean HR, LFnu, LF/HF ratio and increased HFnu, when compared with HFV and HFVil rats (Table 2).

Vildagliptin, dapagliflozin and combined therapy shared a similar efficacy on preventing cardiac arrhythmia in pre-diabetic rats with cardiac I/R injury

Under cardiac I/R, our results demonstrated that the pre-diabetic rats (HFV group) had increased susceptibility to arrhythmia during the reperfusion period, as indicated by decreased time to 1st VT/VF onset (Fig. 3A) and increased arrhythmia score (Fig. 3B), when compared with the NDV group. However, the incidence of VT/VF (Fig. 3C) and VT/VF (Fig. 3D) was not significantly altered between groups in both ischemic and reperfusion periods. The HFV rats also showed a significant reduction in p-Cx43 S₃₆₈ expression (Fig. 3E).

All interventions showed a similar efficacy in increased time to 1st VT/VF onset (Fig. 3A), decreased arrhythmia score (Fig. 3B) and increased p-Cx43 S₃₆₈ expression (Fig. 3E) in the reperfusion period, when compared with the HFV group.

Combined therapy led to a more effective outcome on myocardial infarct size reduction and LV function preservation in pre-diabetic rats with cardiac I/R injury than single regimens

The infarct size, cardiac MDA and apoptotic protein expressions are shown in Fig. 4. HFV rats had a significantly increased myocardial infarct size (Fig. 4A). However, the

HFVil			HFDa			HFDaVil		
Baseline	Ischemia	Reperfusion	Baseline	Ischemia	Reperfusion	Baseline	Ischemia	Reperfusion
227 ± 18 [#]	386 ± 28 [*]	342 ± 25 [*]	212 ± 20 [#]	333 ± 30 [*]	214 ± 20 [†]	212 ± 14 [#]	334 ± 26 [*]	242 ± 16 [†]
106 ± 3 ^{†, #}	67 ± 5 [*]	96 ± 2 [†]	119 ± 4 ^{#, \$}	86 ± 6 [*]	109 ± 5 [†]	120 ± 5 ^{#, \$}	108 ± 9	112 ± 6
7 ± 2 ^{†, #}	13 ± 3 [*]	10 ± 2 [†]	5 ± 2 ^{#, \$}	12 ± 2 [*]	6 ± 1 [†]	5 ± 1 ^{#, \$}	7 ± 3	6 ± 1
143 ± 12	127 ± 16	130 ± 11	136 ± 11	117 ± 13	127 ± 8	132 ± 12	119 ± 9	127 ± 8
7 ± 2	7 ± 3	6 ± 2	7 ± 4	6 ± 2	7 ± 3	6 ± 3	6 ± 2	6 ± 1
6.29 ± 0.28 ^{†, #}	4.49 ± 0.71 [*]	7.16 ± 0.57 [†]	8.07 ± 0.48 ^{#, \$}	5.32 ± 0.37 [*]	7.19 ± 0.39 [†]	7.97 ± 0.74 ^{#, \$}	7.72 ± 0.82	7.94 ± 0.71
6.14 ± 0.31 [#]	4.96 ± 0.22 [*]	6.23 ± 0.28 [†]	6.05 ± 0.43 ^{#, \$}	4.85 ± 0.29 [*]	6.11 ± 0.39 [†]	6.29 ± 0.29 ^{#, \$}	5.71 ± 0.46	6.31 ± 0.25
8 ± 3 [†]	14 ± 4 [*]	9 ± 2 [†]	5 ± 1 ^{#, \$}	10 ± 4 [*]	6 ± 2 [†]	4 ± 2 ^{#, \$}	8 ± 3	6 ± 3
305 ± 26 ^{†, #}	331 ± 12 [*]	310 ± 16 [†]	280 ± 12 ^{#, \$}	320 ± 24 [*]	296 ± 35 [†]	306 ± 17 ^{#, \$}	313 ± 36	287 ± 50
300 ± 21 [#]	157 ± 12 [*]	270 ± 17 [†]	317 ± 24 [#]	239 ± 14 [*]	299 ± 18 [†]	326 ± 19 [#]	285 ± 21	305 ± 23
73 ± 1 ^{†, #}	65 ± 2 [*]	75 ± 1 [†]	82 ± 2 ^{#, \$}	73 ± 2 [*]	79 ± 2 [†]	81 ± 1 ^{#, \$}	78 ± 2	80 ± 1
40.8 ± 2.5 [†]	29.5 ± 1.8 [*]	42.6 ± 1.7 [†]	50.9 ± 3.8 ^{#, \$}	42.0 ± 3.0 [*]	45.3 ± 2.6 [†]	50.8 ± 2.8 ^{#, \$}	46.2 ± 4.4	47.2 ± 4.7

Data are shown as mean ± S.E.M., n = 5–6/group.

*P < 0.05 vs baseline; †P < 0.05 vs ischemia; ‡P < 0.05 vs baseline of NDV; #P < 0.05 vs baseline of HFV; \$P < 0.05 vs baseline of HFVil.

dp/dt_{max} , maximum dp/dt ; dp/dt_{min} , minimum dp/dt ; EDP, end-diastolic pressure; EDV, end-diastolic volume; EF, ejection fraction; ESP, end-systolic pressure; ESV, end-systolic volume; HFD, high-fat diet; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with dapagliflozin and vildagliptin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; HR, heart rate; NDV, normal diet treated with vehicle; P_{max} , maximum pressure; P_{min} , minimum pressure; SV, stroke volume; SW, stroke work.

percentage of area at risk was not different between groups (Fig. 4B). HFV rats had significantly increased cardiac MDA in the ischemic areas (Fig. 4D), with no changes in cardiac MDA at remote areas (Fig. 4E), increased Bax/Bcl-2 ratio (Fig. 4F), without any changes of caspase 3 (Fig. 4G) and increased cleaved caspase 3 (Fig. 4H), when compared with the NDV group.

Although vildagliptin therapy showed a significant reduction in myocardial infarct size (Fig. 4C), cardiac MDA in the ischemic area (Fig. 4D), Bax/Bcl-2 ratio (Fig. 4F) and cleavage caspase 3 (Fig. 4H), dapagliflozin-treated rats had a greater reduction in these parameters than those on vildagliptin therapy. However, the combined therapy showed the greatest efficacy on improving myocardial infarct size (Fig. 4C) and cardiac MDA in the ischemic area (Fig. 4D) in comparison with the NDV, HFV, HFVil and HFDa groups.

The LV function parameters recorded from P–V loops during cardiac I/R are shown in Table 3. At baseline, HFV rats showed a significant reduction in LV function, indicated by reduced end-systolic pressure (ESP), dp/dt_{max} , dp/dt_{min} , SV, ejection fraction (%EF) and SW and also increased heart rate (HR), end-diastolic pressure (EDP), end-systolic volume (ESV) and end-diastolic volume (EDV) in comparison with the baseline in NDV rats. HFVil group had an increase in ESP, dp/dt_{max} , dp/dt_{min} , SV, %EF and SW and a decrease in HR, EDP, ESV and EDV, compared with the baseline measurements of

the HFV rats. Dapagliflozin treatment and combined therapy showed a similar improvement indicated by the restoration of ESP, EDP, dp/dt_{max} , dp/dt_{min} , ESV, EDV, SV, %EF and SW, when compared with HFV and HFVil groups.

During the ischemic period, NDV, HFV, HFVil and HFDa groups showed a significant increase in HR, EDP, ESV and EDV and a decrease in ESP, dp/dt_{max} , dp/dt_{min} , SV, %EF and SW, compared with the baseline data. However, the HFDaVil group had only significantly increased HR, when compared with baseline data. During the reperfusion period, NDV, HFV, HFVil and HFDa groups had a significant decrease in EDP, ESV and EDV and an increase in ESP, dp/dt_{max} , dp/dt_{min} , SV, %EF and SW, compared with the ischemic period. HFDa and HFDaVil groups were associated with a significant reduction of HR in comparison with the ischemic period.

Vildagliptin, dapagliflozin and combined therapy exhibited a similar efficacy on cardiac mitochondrial function, cardiac mitochondrial biogenesis and protein expression in pre-diabetic rats with cardiac I/R injury

The data regarding cardiac mitochondrial function are presented in Fig. 5. The results showed that HFV rats had a significant increase in mitochondrial reactive oxygen species (ROS) production (Fig. 5A)

Figure 5

The effects of vildagliptin, dapagliflozin and combined therapy on cardiac mitochondrial function, cardiac mitochondrial morphology and serum lipid peroxidation in pre-diabetic rats with cardiac I/R injury. (A) Mitochondrial ROS production, $n=6$ per group; (B) mitochondrial membrane potential changes, $n=5-6$ per group; (C) mitochondrial absorbance, $n=5-6$ per group; (D) serum MDA levels, $n=5-6$ per group; and (E) representative cardiac mitochondria morphology by TEM. * $P<0.05$ vs NDV; † $P<0.05$ vs HFV; ‡ $P<0.05$ vs HFVil; and # $P<0.05$ vs HFDa. HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with vildagliptin and dapagliflozin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; MDA, malondialdehyde; MMP, mitochondrial membrane potential; NDV, normal diet treated with vehicle; ROS, reactive oxygen species; TEM, transmission electron microscopy.

and a decrease in mitochondrial red/green fluorescent intensity ratio, indicating mitochondrial depolarization (Fig. 5B), a decrease in mitochondrial absorbance intensity, indicating mitochondrial swelling (Fig. 5C) and increased serum MDA levels (Fig. 5D), compared with the NDV group. Representative electron micrographs showed impaired cardiac mitochondrial morphology in the hearts of HFV rats (Fig. 5E). This cardiac mitochondrial impairment was correlated with a significant reduction in PGC1- α (Fig. 6A), CPT1 (Fig. 6B) and complex I of the electron transport chain (ETC) (Fig. 6C, D, E, F and G).

All interventions had a similar efficacy in leading to improving cardiac mitochondrial function as indicated by reduced mitochondrial ROS production (Fig. 5A), increased mitochondrial red/green fluorescent intensity ratio (Fig. 5B), increased mitochondrial absorbance intensity (Fig. 5C), decreased serum MDA levels (Fig. 5D) and improved cardiac mitochondrial morphology (Fig. 5E), compared with the HFV group. These improvements were also consistent with a significant increase in PGC1- α (Fig. 6A), CPT1 (Fig. 6B) and complex I of the ETC (Fig. 6C).

Combined therapy exerted a greater efficacy on cardiac mitochondrial dynamics than dapagliflozin and vildagliptin in pre-diabetic rats with induced cardiac I/R injury

Cardiac mitochondrial dynamics, including mitochondrial fusion and mitochondrial fission following cardiac I/R injury, are presented in Figs 7 and 8, respectively. HFV rats had decreased expression levels of cardiac mitochondrial MFN2 (Fig. 7A) and cardiac mitochondrial OPA1 (Fig. 7B). The representative bands of MFN2 and OPA1 are shown in Fig. 7C. All interventions showed a similar efficacy in increasing cardiac mitochondrial MFN2 and cardiac mitochondrial OPA1.

Regarding cardiac mitochondrial fission, there was no measurable alteration in cardiac p-DRP1 S₆₁₆ between groups (Fig. 8A), but the level of cardiac p-DRP1 S₆₃₇ was increased (Fig. 8B), compared with the NDV group. However, cardiac DRP1 protein expression was no difference between groups (Fig. 8C). The representative bands of cardiac p-DRP1 S₆₁₆, p-DRP1 S₆₃₇ and cardiac DRP1 protein expression are shown in Fig. 8D. HFV rats had increased expression levels of cardiac mitochondrial DRP1 (Fig. 8E), compared with the NDV group. The representative bands of cardiac

Figure 6

The effects of vildagliptin, dapagliflozin and combined therapy on proteins related to cardiac metabolism and oxidative phosphorylation in pre-diabetic rats with cardiac I/R injury. (A) Myocardial PGC1- α expression in the ischemic area normalized with that in the remote area, $n=6$ per group; (B) cardiac mitochondrial CPT-1 expression in the ischemic area normalized with that in the remote area, $n=6$ per group; (C) cardiac mitochondrial complex I expression in the ischemic area normalized with that in the remote area, $n=6$ per group; (D) cardiac mitochondrial complex II expression in the ischemic area normalized with that in the remote area, $n=6$ per group; (E) cardiac mitochondrial complex III expression in the ischemic area normalized with that in the remote area, $n=6$ per group; (F) cardiac mitochondrial complex IV expression in the ischemic area normalized with that in the remote area, $n=6$ per group; (G) cardiac mitochondrial complex V expression in the ischemic area normalized with that in the remote area, $n=6$ per group; and (H) representative Western blot bands of at PGC1- α , CPT1, complex I, II, III, VI and V in the ischemic area and the remote area. * $P<0.05$ vs NDV; † $P<0.05$ vs HFV. CPT1, carnitine palmitoyltransferase I; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with vildagliptin and dapagliflozin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; NDV, normal diet treated with vehicle; PGC1- α , peroxisome proliferator-activated receptor gamma coactivator 1-alpha; VDAC, voltage-dependent anion channel.

mitochondrial DRP1 protein expression are shown in Fig. 8F. All interventions showed a similar efficacy in increasing cardiac p-DRP1 S₆₃₇ expression, when compared with the HFV group. Although dapagliflozin exerted a more enhanced reduction in cardiac mitochondrial DRP1 expression than vildagliptin, combined therapy showed the greatest efficacy in this reduction, when compared with the NDV, HFV, HFVil and HFDa groups (Fig. 8E).

Discussion

The major findings in this study can be summarized as follows: (1) long-term HFD consumption led to obese-insulin resistance, increased blood pressure, decreased

HRV and impaired LV function; (2) dapagliflozin exerted greater efficacy than vildagliptin in metabolic regulation, HRV, blood pressure and LV function improvements (3) under conditions of cardiac I/R condition, obese insulin-resistant rats had a reduction in the expression of proteins related to the management of cardiac metabolism, cardiac mitochondrial function, complex I of the ETC, cardiac mitochondrial fusion and an increase in the expression of proteins related to cardiac mitochondrial fission and myocyte apoptosis, leading to larger infarct size than that seen in ND rats; (4) although dapagliflozin had more beneficial effects on cardioprotection than vildagliptin in pre-diabetic rats with initiated cardiac I/R injury, a combined therapy of the two agents demonstrated greater

Figure 7

The effects of vildagliptin, dapagliflozin and combined therapy on cardiac mitochondrial fusion in pre-diabetic rats with cardiac I/R injury. (A) Cardiac mitochondrial MFN2 expression in the ischemic area normalized with that in the remote area, $n=5-6$ per group; (B) cardiac mitochondrial OPA1 expression in the ischemic area normalized with that in the remote area, $n=5-6$ per group; and (C) representative Western blot bands of MFN2 and OPA1 using cardiac mitochondrial fraction as a samples. * $P<0.05$ vs NDV; and $^{\dagger}P<0.05$ vs HFV. HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with vildagliptin and dapagliflozin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; MFN2, mitofusin 2; NDV, normal diet treated with vehicle; OPA1, optic atrophy 1; VDAC, voltage-dependent anion channel.

cardioprotective effects than either of the single regimens. Evidence suggests that this outcome was due to markedly decreased mitochondrial fission and cardiac oxidative stress, leading to the largest reduction in myocardial infarct size in HFDaVil rats with cardiac I/R injury (Fig. 9).

Although these drugs are for the treatment of type 2 diabetes, we believe that early treatment and prevention since pre-diabetic state is crucial and have been investigating the roles of pharmacological interventions as well as device intervention in pre-diabetic conditions like the obese insulin-resistant model used in this study for many years (Pipatpiboon *et al.* 2013, Apaijai *et al.* 2014, 2016, Chinda *et al.* 2014, Tanajak *et al.* 2017). In addition, due to the fast growing number of obese insulin-resistant population worldwide, we believe that early interventions in this group prior to the development into type 2 diabetes will provide impacts on therapeutic strategies as well as possible reduction in economic burden. Moreover, there are many studies investigating the effects of the drugs in type 2 diabetes, but only a few that investigates these effects under pre-diabetic obese insulin-resistant condition as well as their cardioprotective effects. Furthermore, regarding dapagliflozin, previous studies demonstrated that it not only exerts the beneficial effects on glucose lowering in diabetes models, but also has the pleiotrophic effects such as reduced body weight, reduced blood pressure, improved metabolic regulation, renoprotection and neuroprotection in non-diabetic models (Han *et al.* 2008, Lee *et al.* 2017a, Lundkvist *et al.* 2017, Sa-Nguanmoo *et al.* 2017). For these, we aimed to investigate the cardioprotective efficacy of dapagliflozin in comparison to vildagliptin and combined therapy in a pre-diabetic obese insulin-resistant model, which has never been investigated. We believe that the findings from this study could provide insights for future clinical application in obese insulin-resistant patients especially those with cardiac ischemia/reperfusion injury.

In the pre-diabetic condition, dapagliflozin showed a greater efficacy in restoring metabolic regulation, HRV and LV function when compared with vildagliptin. Also only rats treated with dapagliflozin showed a significant reduction in SBP and mean arterial pressure, which was not found in vildagliptin-treated rats. Previous studies demonstrated that dapagliflozin therapy caused a reduction in blood pressure due to diuretic effects (Lambers Heerspink *et al.* 2013, Weber *et al.* 2016). These findings could be deemed similar to our results as our data also indicated an increase in the level of urine excretion in dapagliflozin-treated rats, which could play an important role in blood pressure reduction and weight reduction.

HRV has been identified as a powerful independent predictor of increasing mortality in post myocardial infarction patients (Kleiger *et al.* 1987). HRV became acknowledged as an indicator of cardiac sympathovagal imbalance in numerous clinical studies to identify patients

Figure 8

The effects of vildagliptin, dapagliflozin and combined therapy on cardiac mitochondrial fission in pre-diabetic rats with cardiac I/R injury. (A) Myocardial p-DRP1 Ser₆₁₆ expression in the ischemic area normalized with that in the remote area, $n=5-6$ per group; (B) myocardial p-DRP1 Ser₆₃₇ expression in the ischemic area normalized with that in the remote area, $n=5-6$ per group; (C) myocardial DRP1 expression in the ischemic area normalized with that in the remote area, $n=6$ /group; (D) representative Western blot bands of p-DRP1 Ser₆₁₆, p-DRP1 Ser₆₃₇ using myocardial tissues as samples; (E) cardiac mitochondrial DRP1 expression in the ischemic area normalized with that in the remote area, $n=6$ per group and (F) representative Western blot bands of DRP1 using cardiac mitochondrial fraction as a samples. * $P<0.05$ vs NDV; † $P<0.05$ vs HFV; ‡ $P<0.05$ vs HFVil; and # $P<0.05$ vs HFDa. DRP1, dynamin-related protein 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HFDa, high-fat diet treated with dapagliflozin; HFDaVil, high-fat diet treated with vildagliptin and dapagliflozin; HFV, high-fat diet treated with vehicle; HFVil, high-fat diet treated with vildagliptin; NDV, normal diet treated with vehicle; p-DRP1 Ser₆₁₆, phosphorylation of DRP1 at serine616; p-DRP1 Ser₆₃₇, phosphorylation of DRP1 at serine637; VDAC, voltage-dependent anion channel.

who have an increased risk of cardiovascular mortality in populations with and without known CVD (Bigger *et al.* 1992, La Rovere *et al.* 1998, Liao *et al.* 2002, Hillebrand *et al.* 2013). Our study demonstrated that pre-diabetic rats had impaired HRV due to the shift of cardiac autonomic balance towards sympathetic dominance. In the present study, dapagliflozin exerted more effective HRV restoration than the vildagliptin treatment, suggesting that dapagliflozin therapy exerts a higher efficacy on improved cardiac autonomic control in the pre-diabetic condition than vildagliptin.

Fatty acid oxidation is a major source of ATP from cardiac energy metabolism and the alteration of cardiac metabolism can reduce cardiac efficiency (Lopaschuk *et al.* 2010). Cardiac mitochondrial metabolism-related proteins PGC1- α and CPT1 are essential proteins for the regulation of cardiac fatty acid oxidation (Duncan 2011, Lucas *et al.* 2016). Our results showed that PGC1- α and CPT1 levels were decreased in the I/R hearts of obese insulin-resistant rats. Although previous studies demonstrated the reduction in myocardial PGC1- α and CPT-1 protein expression in genetic obesity model (Young *et al.* 2002) and HFD-induced obese models (Neves *et al.* 2014, Tanajak *et al.* 2016, 2017), the alteration in cardiac PGC1- α protein expression and cardiac mitochondrial CPT-1 protein expression in HFD-induced obese insulin-resistant with myocardial I/R injury model

compared with lean model has never been reported. This study is the first to demonstrate these impairments. In addition, the expression of cardiac mitochondrial complex I of the ETC and cardiac mitochondrial function were reduced in these HFV rats after I/R injury. Therefore, reduced levels of cardiac PGC1- α , cardiac mitochondrial CPT1, cardiac mitochondrial complex I of the ETC and decreased cardiac mitochondrial function, may all be responsible for the reduction in cardiac energy metabolism in cases of I/R injury. Regarding pharmacological interventions in this study, we found that vildagliptin, dapagliflozin and combined therapy, showed a similar efficacy on increasing the expression of cardiac mitochondrial metabolism-related proteins PGC1- α and CPT1, the cardiac mitochondrial complex I of the ETC and also improving cardiac mitochondrial function. Regarding protein-related cardiac arrhythmias Cx43 and arrhythmogenesis profiles, a decreasing in gap junction protein p-Cx43 S₃₆₈ expression, decreasing in time to first VT/VF onset and increasing arrhythmia score during I/R injury were found in HFV rats. All interventions gave a similar efficacy by increasing gap junction protein p-Cx43 S₃₆₈ expression. This could be mainly responsible for reducing the arrhythmia vulnerability during I/R injury in these rats.

An inhibition of cardiac mitochondrial fission could result in cardioprotection against myocardial I/R injury

Figure 9

Diagram summarizing the effects of high-fat diet consumption, vildagliptin, dapagliflozin and combined therapy in pre-diabetic rats and pre-diabetic rats with cardiac I/R injury. CPT1, carnitine palmitoyltransferase I; I/R, ischemia/reperfusion; LV, left ventricle; PGC-1 α , peroxisome proliferator-activated receptor gamma coactivator 1- α . A full color version of this figure is available at <http://dx.doi.org/10.1530/JOE-17-0457>.

(Ong *et al.* 2010), and this has also been seen to enhance survival in a murine cardiac arrest model (Sharp *et al.* 2015). Regarding the results of cardiac mitochondrial dynamics in this study, we found that cardiac mitochondrial fission was increased and mitochondrial fusion was decreased in HFV rats with I/R injury. Although all intervention arms had a similar efficacy on increasing cardiac mitochondrial fusion after cardiac I/R, dapagliflozin therapy had a higher efficacy than vildagliptin therapy, the greater impact being identified by a decrease in cardiac mitochondrial fission. However, the combined therapy exerted the greatest efficacy on reducing cardiac mitochondrial fission after cardiac I/R. This study is the first to demonstrate the efficacy of pharmacological intervention (vildagliptin and dapagliflozin) on cardiac mitochondrial dynamic improvements in pre-diabetic rats with cardiac I/R injury. Although dapagliflozin showed a higher efficacy in decreasing cardiac mitochondrial fission than vildagliptin, a combination therapy of the 2 agents showed the highest efficacy as regards cardiac mitochondrial fission reduction through a marked reduction in cardiac mitochondrial DRP1 expression. However, all pharmacological intervention gave a similar efficacy in

cardiac mitochondrial fusion through increasing cardiac mitochondrial MFN2 and OPA1 expression. These findings indicate that combined therapy provides a better restoration of cardiac mitochondrial dynamics than either of the single regimens. In addition, the combined therapy provided the most effective outcome for cardioprotection following myocardial I/R potentially through a reduction in both mitochondrial fission and cardiac MDA levels being seen in the ischemic areas. In this study, cardiac mitophagy was not investigated as a further determinant. Since mitophagy plays an important role in controlling the mitochondrial fragmentation (Burman *et al.* 2017, Meyer *et al.* 2017) and also potentially the determinant of the cardiac function in this case (Li *et al.* 2016, Feng *et al.* 2017, Lee *et al.* 2017b, Zhou *et al.* 2017), future studies are needed to confirm the findings in the present study.

In conclusion, dapagliflozin exerted a greater efficacy on the regulation of metabolism, the balance of the cardiac autonomic system, reduction of blood pressure and improvement in LV function, compared to vildagliptin in pre-diabetic rats. However, under conditions of cardiac I/R, although dapagliflozin had greater efficacy than vildagliptin, combined therapy provided the

more enhanced cardioprotective benefits by reducing myocardial infarct size and improving LV function. The outstanding efficacy of the combined therapy potentially through a reduction in mitochondrial fission and cardiac MDA levels.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-17-0457>.

Declaration of interest

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

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Author contribution statement

Conception and design of experiments: S C and N C. Conduction of experiments: P T, P S, S S and S T. Data analysis: P T, P S, S S, S T, N S, S C and N C. Manuscript construction: P T, S C and N C.

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