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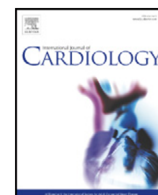

Cardioprotective effect of dipeptidyl peptidase-4 inhibitor during ischemia–reperfusion injury

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ABSTRACT

Background: Dipeptidyl peptidase-4 (DPP-4) inhibitor is a new anti-diabetic drug for type-2 diabetes mellitus patients. Despite its benefits on glycemic control, the effects of DPP-4 inhibitor on the heart during ischemia–reperfusion (I/R) periods are not known. We investigated the effect of DPP-4 inhibitor on cardiac electrophysiology and infarct size in a clinically relevant I/R model in swine and its underlying cardioprotective mechanism.
Methods: Fourteen pigs were randomized to receive either DPP-4 inhibitor (vildagliptin) 50 mg or normal saline intravenously prior to a 90-min left anterior descending artery occlusion, followed by a 120-min reperfusion period. The hemodynamic, cardiac electrophysiological and arrhythmic parameters, and the infarct size were determined before and during I/R. Rat cardiac mitochondria were used to study the protective effects of DPP-4 inhibitor on cardiac mitochondrial dysfunction caused by severe oxidative stress induced by H_2O_2 to mimic the I/R condition.
Results: Compared to the saline group, DPP-4 inhibitor attenuated the shortening of the effective refractory period (ERP), decreased the number of PVCs, increased the ventricular fibrillation threshold (VFT) during the ischemic period, and also decreased the infarct size. In cardiac mitochondria, DPP-4 inhibitor decreased the reactive oxygen species (ROS) production and prevented cardiac mitochondrial depolarization caused by severe oxidative stress.
Conclusions: During I/R, DPP-4 inhibitor stabilized the cardiac electrophysiology by preventing the ERP shortening, decreasing the number of PVCs, increasing the VFT, and decreasing the infarct size. This cardioprotective effect could be due to its prevention of cardiac mitochondrial dysfunction caused by severe oxidative stress during I/R.

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1. Introduction

Diabetes mellitus has been an important health problem in most nations with the number of patients dramatically soaring and expected to reach 366 million by the year 2030 [1]. Patients with type-2 diabetes mellitus (T2DM) have been shown to have a 2- to 4-fold higher risk in coronary heart disease and stroke mortality [2–6], and have a worse prognosis after cardiovascular events [7–9]. Although several new anti-diabetic drugs have been discovered in the past decades, the therapies have been limited by their adverse effects such as weight gain, hypoglycemia, fluid retention [10], and an unexpected cardiovascular risk [11–13]. Therefore, new anti-diabetic drugs that could control hyperglycemia and reduce the risk of cardiovascular events are of potential benefits to T2DM patients.

In the past few years, a potent dipeptidyl peptidase-4 (DPP-4) inhibitor, which is a novel anti-diabetic drug, has been shown to be effective in treating T2DM patients. Its action is to inhibit the proteolytic enzyme DPP-4 activity, resulting in postponing the degradation of glucagon-like peptide-1 (GLP-1), thus improving glycemic control [14,15]. Although previous studies demonstrated the cardioprotective actions of GLP-1 in an ischemic heart model of ex vivo isolated rodent Langendorff heart [16,17], in vivo rats, rabbits, canine, and swine [18–21], as well as in acute myocardial infarction patients [22], reports on the cardioprotective effect of DPP-4 inhibitor are scant and controversial [23–26]. Furthermore, the effect of DPP-4 inhibitor on cardiac electrophysiology during ischemia–reperfusion (I/R) has never been investigated.

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The purpose of this study was to investigate the effect of vildagliptin, a DPP-4 inhibitor, on cardiac electrophysiology and infarct size in a clinically relevant I/R model in swine. We hypothesized that vildagliptin can attenuate the occurrence of cardiac arrhythmias, increase the ventricular fibrillation threshold (VFT), improve defibrillation efficacy by lowering the defibrillation threshold (DFT), and reduce the infarct size during I/R in the swine heart. To study the cardioprotective mechanism, we determined the effect of vildagliptin in isolated rat's cardiac mitochondria. We tested the hypothesis that the cardioprotective mechanism of vildagliptin is via its prevention of cardiac mitochondrial dysfunction caused by severe oxidative stress during I/R.

2. Materials and methods

2.1. Animal preparation

All experiments were approved by the Institutional Animal Care and Use Committees of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Pigs were anesthetized by intramuscular injection of a combination of atropine (0.04 mg/kg), zoletil® (4.4 mg/kg) and xylazine (2.2 mg/kg). After endotracheal intubation, anesthesia was maintained by 1.5–3.0% isoflurane delivered in 100% oxygen. Surface electrocardiogram (lead II), femoral arterial blood pressure (BP), heart rates (HR), core body temperature as well as blood gases and electrolytes were continuously monitored to maintain a normal physiological condition. Platinum coated titanium coil electrodes (34- and 68-mm) were advanced into the right ventricular apex (RV) and junction between right atrium and superior vena cava, respectively, to deliver electrical stimulus during VFT and DFT determinations [27]. After a median sternotomy, two pacing electrodes were attached to the epicardium at the right ventricular outflow tract (RVOT) and left ventricular apex (LV) to evaluate the effective refractory period (ERP) and diastolic pacing threshold (DPT) at each site. The electrode at the tip of the endocardial RV apex catheter was also used to determine the ERP and DPT at this site.

2.2. Experimental protocols

Fourteen domestic pigs (25 to 30 kg) were randomly divided into 2 groups (n = 7/group). The first group was assigned to receive 30 ml of normal saline solution and the second group received vildagliptin (prepared by dissolving 50-mg vildagliptin in 30-ml saline solution). Both normal saline solution and vildagliptin (2 mg/Kg) were administered intravenously at a rate of 1.0 ml/min prior to the left anterior descending artery (LAD) occlusion. Hemodynamic and cardiac electrophysiological parameters including HR, systolic (SBP) and diastolic blood pressure (DBP), DPT, ERP, corrected QT interval (QT_C), VFT and DFT were determined at the beginning of the study as a baseline. Myocardial ischemia was induced by LAD occlusion at 5 cm above the distal end [28]. During the first 60 min of occlusion, if spontaneous ventricular fibrillation (VF) occurred, the defibrillation shock was delivered to determine the DFT. On the other hand, if VF did not occur, it was electrically induced with 50-Hz alternating current. Both VFT and DFT were determined using a three-reversal up/down protocol [28]. After 90 min of occlusion, LAD ligation was released to promote reperfusion for 120 min. All parameters were determined again at the end of the reperfusion. Ventricular arrhythmia, e.g. ventricular premature contractions (PVCs), ventricular tachycardia (VT) and spontaneous VF, was recorded throughout the experiment.

2.3. Diastolic pacing threshold (DPT) determination

A train of 10 S1 stimuli was delivered via the electrode at the tip of RV catheter. Current strength was begun with 0.1 mA and was increased in 0.1-mA steps until all stimuli in a train elicited a ventricular response (capture) [28]. The minimum current strength which captures ventricular response was defined as the DPT.

2.4. Effective refractory period (ERP) determination

An S2 stimulus (2× DPT strength) was introduced in late diastole of the last S1 beat of a train of 10 S1 to elicit a capture. S1–S2 coupling interval was decreased in 10-ms steps until S2 failed to elicit a capture. ERP was defined as the longest S1–S2 interval which S2 stimulus failed to capture [28].

2.5. Ventricular fibrillation threshold (VFT) determination

The interval between the last S1 and the mid T-wave was determined for 3 times. An average was used as a coupling interval between the last S1 and S2 shock. VFT was performed by delivering S2 shocks starting at 100 V. If this shock induced VF, the decrement of 10-V step was used for each successive shock until VF was no longer induced. If the 100-V S2 shock did not induce VF, the increment of 10-V step was used for each successive shock until VF was induced. VFT was defined as the lowest shock strength that successfully induced VF [28].

2.6. Defibrillation threshold (DFT) determination

Defibrillation shock was delivered after 10 s of VF to determine the DFT using a three-reversal up/down protocol [28]. However, if the tested shock failed to defibrillate, a rescue shock (600–700 V) was delivered to successfully defibrillate the heart. The DFT was defined as the lowest shock strength required for successful defibrillation. A 4-minute interval was allowed between each VF induction episode to set the heart back to physiologic condition [28].

2.7. Infarct size determination

The infarct size was assessed with 0.5% Evans Blue and 1.0% Triphenyltetrazolium Chloride (TTC) staining as previously described [28]. In brief, at the end of the study, the LAD was re-occluded at the exact same location as during ischemia. Evans Blue was infused into the left and right coronary arteries to evaluate the area at risk (AAR). After being frozen overnight, the heart was cut into 5-mm thick slices perpendicular to the LAD from apex to the occlusion site. Each slice was incubated in TTC for 15 min to discriminate the infarct tissues from the viable myocardium. After overnight fixation with 4% paraformaldehyde, each slice was photographed with a digital camera. An area measurement was performed using Image Tool software version 3.0.

2.8. Histological analysis

Both infarct and normal myocardium were fixed with 4% neural buffered formaldehyde for 24 h at room temperature, followed by embedding in paraffin wax, and slicing into 5-μm slices for subsequent Hematoxylin–Eosin staining [29,30]. The infarct tissues were evaluated for microscopic changes using the Lodge–Patch classification [31–33].

2.9. Isolated cardiac mitochondria study protocol

Male Wistar rats (300–350 g) were used for cardiac mitochondrial isolation as described previously [34]. H_2O_2 (2 mM, incubated for 5 min) was used to induce oxidative stress in cardiac mitochondria to mimic I/R condition [34]. Isolated cardiac mitochondria were divided into 6 groups (n = 5/group): 1) Control, 2) Mitochondria treated with H_2O_2, 3) Mitochondria treated with vildagliptin for 30 min, 4) Mitochondria pretreated with vildagliptin for 5 min followed by H_2O_2 treatment, 5) Mitochondria pretreated with vildagliptin for 15 min followed by H_2O_2 treatment, 6) Mitochondria pretreated with vildagliptin for 30 min followed by H_2O_2 treatment. Vildagliptin at doses of 0.33 mM, and 3.30 mM were used in this study.

The measurement of cardiac mitochondrial reactive oxygen species (ROS) production and mitochondrial membrane potential changes ($\Delta\Psi_M$) was determined in all groups as previously described [34]. In short, dichlorohydro-fluorescein diacetate dye was used to determine the level of ROS production in cardiac mitochondria. The ROS level was expressed as arbitrary units of fluorescence intensity determined at $\lambda_{excitation}$ 485 nm and $\lambda_{emission}$ 530 nm [34]. JC-1 was used to determine the change of cardiac mitochondrial membrane potential at $\lambda_{excitation}$ 485 nm and $\lambda_{emission}$ 530 nm for green and $\lambda_{emission}$ 590 nm for red [34]. Cardiac mitochondrial depolarization was indicated by a decrease in the red/green fluorescence intensity ratio.

2.10. Statistical analysis

Data were expressed as mean ± SEM. Statistical comparison of cardiac electrophysiological, hemodynamic, and arrhythmic parameters as well as cardiac mitochondria results was performed with the Student's *t* test. Chi-square test was performed to compare VT/VF incidence, and comparison of the infarct size was analyzed using Mann–Whitney's *U* test. All statistical analysis was performed with SPSS version 10.0. A p-value less than 0.05 was considered significant.

3. Results

The hemodynamic parameters including HR, SBP, DBP and cardiac electrophysiological parameters including QT_C, QRS complex, and DPT were not significantly different between the saline-treated group and the vildagliptin-treated group during baseline, ischemia, and reperfusion periods (Table 1). Pretreatment with vildagliptin significantly increased both VFT energy and VFT voltage at ischemic period compared with the saline-treated group (Fig. 1). However, there was no difference between the DFT of the vildagliptin-treated group and the saline-treated group at any time period (Fig. 2). For the ERP, no differences were found among ERPs recorded at three sites at baseline and reperfusion periods in both saline and vildagliptin treated groups (Fig. 3A and 3C). However, during ischemia the ERP at the LV epicardium (i.e. ischemic site) was significantly shortened, compared to the other two sites in the saline-treated group (Fig. 3B), thus creating the dispersion of the ERP in the heart during this period. In the

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