**EXPERIMENTAL STUDY**

**Linagliptin Suppresses Electrical and Structural Remodeling in the Isoproterenol Induced Myocardial Injury Model**

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**Summary**

The effect of DPP-4 inhibitor on the electrical and structural remodeling in myocardial injury has not been evaluated. We hypothesized that linagliptin, DPP-4 inhibitor, suppresses myocardial remodeling in the isoproterenol (ISP)-induced myocardial injury model.

Sprague-Dawley rats were assigned to 3 groups: 1) sham group, 2) ISP group (subcutaneous ISP injection of 70 mg/kg), and 3) ISP + linagliptin (ISP + Lin) (5 mg/kg/day, p.o.) group. Serum was sampled on day 1 (acute phase) and day 7 (sub-acute phase) to evaluate derivatives of reactive oxidative metabolites (d-ROMs). The electrophysiological study was performed in sub-acute phase for the evaluation of the ventricular effective refractory period (VERP) and monophasic action potential duration (MAPD). The VERP and MAPD were markedly prolonged in the ISP group in comparison with the sham (MAPD20: 14 ± 6 versus 11 ± 3 ms, MAPD90: 57 ± 8 versus 44 ± 7 ms, VERP: 74 ± 22 versus 38 ± 10 ms, P < 0.05). In contrast in the ISP + Lin group, such prolongations were suppressed, and the parameters were shorter than the ISP group (MAPD20: 9 ± 2 ms, MAPD90: 35 ± 6 ms, VERP: 52 ± 13 ms, P < 0.05). ISP treatment induced myocardial injury. The injured area was reduced in the ISP + Lin group in comparison with the ISP group (P < 0.05). Serum d-ROMs level in acute phase was higher in ISP group than the other 2 groups (sham: 214 ± 55 versus ISP: 404 ± 45 versus ISP + Lin: 337 ± 20 U.CARR, P < 0.05).

Linagliptin suppressed structural and electrical changes, possibly through the antioxidative effect, in this myocardial injury model.

**Key words:** DPP-4 inhibitor

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Linagliptin, Dipeptidyl peptidase-4 (DPP-4) inhibitor is an incretin-based anti-diabetic medicine that is widely used in clinical practice to control blood sugar level in patients with diabetes mellitus.1 As the mechanism of the effect of DPP-4 inhibitors, increased availability of intrinsic glucagon like protein-1 (GLP-1) is considered to play a main role through the stimulation of glucose depended insulin release.1 Because GLP-1 receptors are known to express in various tissues, such as endocellular cells, smooth muscle, myocardium, and pancreas β-cell, pleiotropic effect might be expected in a usage of DPP-4 inhibitors. By referring to several recent studies, the possibility of cardioprotective and/or anti-fibrotic effects have been documented in clinical cases, although the precise mechanism is still unclear.3,4

Isoproterenol (ISP) is a synthetic catecholamine and is one of the strong β-adrenoceptor agonists. The ISP induced myocardial injury model in rat is one of the most popular experimental models used mimicking acute myocardial infarction. High-dose ISP injection has been known to induce myocardial necrosis by producing highly cytotoxic free radical.4,5 Such pathological and morphological alteration are predominantly observed in endocardial area, so that this model was considered comparable with myocardial infarction in human. In such myocardial injury, hyperoxidative stress (i.e., increased reactive oxidative species (ROS)) is considered to play an important role causing ventricular remodeling.9,10 We have previously documented that primary oxidative stress can cause ventricular electrical and structural remodeling in the glutathione depleted knockout mice models.12,13 So that similar ventricular remodeling will be expected in the ISP-induced myocardial injury model. Because some of the DPP-4 inhibitors exhibited attenuation of the oxidative stress in cardiac ischemia reperfusion injury rat model,14,15 DPP-4 inhibitors may suppress the myocardial remodeling.
through the suppression of hyperoxidative stress.

In the present study, we aimed to test the effect of li-nagliptin on the ventricular structural and electrical remodeling in the ISP induced myocardial injury model.

**Methods**

**Subject:** Eight weeks old Male Sprague-Dawley rats (CLEA JAPAN, Inc, Tokyo, Japan) were kept in the departmental animal house under controlled conditions of temperature at 25 ± 2°C, relative humidity of 60 ± 5%, and standardly controlled light. We have determined the body weights of rats similarly to the previous study. They were fed with standard food pellets and water ad libitum. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the Ethics Committee of Kitasato University School of Medicine.

**Experimental protocol and grouping:** Myocardial injury was induced by subcutaneous (s.c.) injection of ISP (70 mg/kg) as previously reported, and this day of injection was defined as day 0 in the protocol. A total of 81 rats were used in this study, and they were assigned to 3 groups as follows (Figure 1): 1) sham group (n = 22): rats were injected with saline (1 mL) on day 0, and the buffer water was orally administrated every day (1 mL/kg/day) using gastric tube starting at 7 days preceding the saline injection (day -7) and continued until day 7 in the protocol, i.e., 14 days in total, 2) ISP group (n = 39): rats received a subcutaneous ISP injection (70 mg/kg) on day 0, and the buffer water was orally administered just similar to the sham group, and 3) ISP + linagliptin (ISP + Lin) group (n = 20): rats suffered from ISP injection on day 0, and linagliptin (5 mg/kg/day in 1 mL buffer water) was orally administrated every day using gastric tube starting at day -7 and continued until day 7.

**Measurement of myocardial injury and DPP-4 activity:** As a marker of myocardial injury, the levels of troponin I were measured by ELISA (rat Cardiac TnI ELISA Kit; Life Diagnostics, Inc.) in the serums sampled at day 1 as the sample in the acute phase, and day 7 as the sample in the sub-acute phase. The DPP-4 activity was measured by ELISA (BioVision Incorporated, Milpitas Boulevard, Milpitas, USA) in the serum sampled at day 7.

**Histology and measurement of myocardial fibrosis:** The heart was sampled for histological evaluation in selected rats at the end of the protocol, i.e., 8 in the sham, 17 in the ISP and 9 in the ISP + Lin groups, respectively. The heart was transversely sliced and fixed in 10% formalin, embedded in paraffin. Transverse sections of the sliced left ventricle were stained with Hematoxylin and Eosin (H.E.) staining for the morphological analysis and with Azan staining for the evaluation of tissue fibrosis. The fibrotic area was quantified in each slice by digitized image analyzer (Lumina vision co ltd., Tokyo, Japan).

**Measurement of heart rate and blood pressure:** Heart rate and systolic BP was evaluated with a tail cuff and a pneumatic pulse transducer (BP-98; Softron, Tokyo, Japan).

**Electrophysiological study:** At the end of the protocol before the sacrifice, the electrophysiological evaluation was performed under the anesthesia using sodium pentobarbital (10-50 mg/kg) with controlled ventilation. The heart was exposed by the median sternotomy, and the electrical stimulations and/or recordings were performed through a pair of plunged platinum needle electrodes (φ 0.1 mm) as previously described. The heart rate was controlled not to exceed 400 beats/minute by additional intravenous anesthesia with sodium pentobarbital. The
analog signals were converted into digital signals at a sampling frequency of 1,000 Hz (PowerLab/SP; ADInstruments Pty Ltd, Bella Vista, NSW, Australia) and stored on a computer hard disk. The bandpass filter was set at 50-300 Hz for standard cardiac electrogram recording and at open -300 Hz for recording the monophasic waveform (MAP). To evaluate the ventricular effective refractory period (VERP), a single extrastimulus was delivered after 8 basic stimuli with a cycle length of 140, and 120 msec. The coupling interval of the extrastimulus was shortened in 2-msec steps until it reached ventricular refractoriness. To standardize the influence of activation cycle length, MAP was evaluated using the last beat of 8 consecutive passed beats with fixed cycle length of 140 and 120 msec. The MAP duration (MAPD) was determined as the interval between the onset of the MAP trace and the 20% (MAPD20) and 90% (MAPD90) repolarization times. The electrophysiological study was performed in selected mice (8 in the sham, 14 in the ISP, and 10 in the ISP + Lin groups) to avoid the influence of mechanical injury on the data of each group. Body weight did not differ among the groups (ISP: 380 ± 22 g versus ISP + Lin: 378 ± 22 g versus sham: 395 ± 36 g; Table), but the heart weight and heart weight - body weight (HW/BW) ratio were higher in the ISP and the ISP+Lin groups than the sham group. HW and HW/BW ratio were lower in the ISP + Lin group than the ISP group (HW, ISP: 1.38 ± 0.12 g versus ISP + Lin: 1.30 ± 0.15 g versus sham: 1.18 ± 0.09 g, HW/BW, ISP: 3.38 ± 0.38 g versus ISP + Lin: 3.26 ± 0.30 g; Table) (Fig. 2). In contrast, in the sub-acute phase, the level of troponin I did not differ among the groups (ISP: 0.15 ± 0.03 ng/mL versus ISP + Lin: 0.03 ± 0.01 ng/mL, NS; Figure 2). In contrast, in the sub-acute phase, the level of troponin I did not differ among the groups (ISP: 0.15 ± 0.03 ng/mL versus ISP + Lin: 0.03 ± 0.01 ng/mL, NS; Figure 2).

**Evaluation of oxidative stress:** The serum levels of derivatives of reactive-oxygen metabolites (d-ROMs) were measured using the Free Radical Analytical System 4 (FRAS4; H&D srl, Parma, Italy) on acute and sub-acute phases. Measurement of d-ROMs levels is defined as CARRATELLI UNITS (U.CARR) according to previous correlation. The specific activity of DHE signals for O2- was confirmed by pre-incubation with their inhibitor, polyethyleneglycol-superoxide dismutase (PEG-SOD; 500 U/mL; Sigma). DHE images were obtained using an excitation filter of 488 nm, and emission filter of 580 nm by a laser-scanning confocal microscope (LMS710; Carl Zeiss MicroImaging Co, Ltd, Oberkochen, Germany). Fluorescence was quantified by ZEN 2008 image analysis software (Carl Zeiss MicroImaging Co, Ltd).

**Statistical analysis:** Statistical analysis was performed with JMP 11.2 (SAS Institute, Cary, North Carolina, USA) statistical software package. Continuous variables are presented as the mean ± standard deviation, and were compared using Student’s t test or the Mann-Whitney U test. Discontinuous variables are presented as numbers or percentages and were compared using the chi-square test.

Multi-factor statistical analysis was performed by using Tukey-Kramer test. A P value of < 0.05 was considered statistically significant.

**Results**

**Heart and body weight:** Heart and body weights were measured at the end of the protocol and the Table shows the data of each group. Body weight did not differ among the groups (ISP: 380 ± 22 g versus ISP + Lin: 378 ± 22 g versus sham: 395 ± 36 g; Table), but the heart weight and heart weight - body weight (HW/BW) ratio were higher in the ISP and the ISP+Lin groups than the sham group. HW and HW/BW ratio were lower in the ISP + Lin group than the ISP group (HW, ISP: 1.38 ± 0.12 g versus ISP + Lin: 1.30 ± 0.15 g versus sham: 1.18 ± 0.09 g, HW/BW, ISP: 3.38 ± 0.38 g versus ISP + Lin: 3.26 ± 0.30 g versus sham: 3.00 ± 0.32 g; Table).

**Heart rate and systolic blood pressure:** Heart rate did not differ among the groups (ISP: 405.4 ± 32.0 bpm versus ISP + Lin: 374.1 ± 42.4 bpm versus sham: 374.1 ± 45.5 bpm, NS; Table I). Systolic blood pressure was significantly lower in the ISP and ISP + Lin groups than in the sham group (ISP: 113.8 ± 8.0 mmHg versus ISP + Lin: 121.0 ± 13.3 mmHg versus sham: 136.8 ± 19.6 mmHg, P < 0.05; Table).

**Level of myocardial injury:** In the acute phase, the serum level of troponin I was lower in the ISP + Lin group than the ISP group (ISP: 12.18 ± 4.97 ng/mL versus ISP + Lin: 8.51 ± 3.06 ng/mL versus sham: 0.03 ± 0.01 ng/mL, P < 0.05; Figure 2). In contrast, in the sub-acute phase, the level of troponin I did not differ among the groups (ISP: 0.15 ± 0.03 ng/mL versus ISP + Lin: 0.03 ± 0.01 ng/mL versus sham: 0.03 ± 1.01 ng/mL, NS; Figure 2).

**Level of DPP-4 activity and free blood sugar:** The serum DPP-4 activity was lower in the ISP + Lin group than the sham and ISP groups (ISP: 2718 ± 239 pmol/L versus sham: 0.0392*; Figure 1). In contrast, the sub-acute phase, the level of troponin I did not differ among the groups (ISP: 0.15 ± 0.03 ng/mL versus ISP + Lin: 0.03 ± 0.01 ng/mL versus sham: 0.03 ± 1.01 ng/mL, NS; Figure 2).

- **Table.** Effect of Linagliptin on Heart Weight, Body Weight, Hemodynamic Data, and Serum Free Blood Sugar Level in Isoproterenol Induced Myocardial Injury in Rats

|                          | Sham   | ISP    | ISP + Lin | P value | Sham versus ISP | Sham versus ISP + Lin | ISP versus ISP + Lin |
|--------------------------|--------|--------|-----------|---------|-----------------|-----------------------|----------------------|
| Heart weight (HW), (g)    | 1.18 ± 0.09 | 1.38 ± 0.12 | 1.30 ± 0.15 | 0.0003* | 0.0010          | 0.0182*               | 0.0392*              |
| Body weight (BW), (g)     | 395 ± 36 | 380 ± 22 | 378 ± 22  | 0.1308  | 0.0151          | 0.0366*               | 0.4701               |
| HW/BW ratio              | 3.00 ± 0.32 | 3.66 ± 0.38 | 3.38 ± 0.26 | < 0.0001* | 0.0035*       | 0.4117                 | 0.0728               |
| Heart rate, (bpm)         | 374.1 ± 45.5 | 405.4 ± 32.0 | 359.2 ± 42.4 | 0.01094* | 0.04426        | 0.9804                 | 0.0052*              |
| Systolic blood pressure, (mmHg) | 136.8 ± 19.6 | 113.8 ± 8.0 | 121.0 ± 13.3 | 0.0074* | 0.003*          | 0.0277*                | 0.3442               |
| Diastolic blood pressure, (mmHg) | 83.3 ± 10.4 | 70.1 ± 11.7 | 65.9 ± 14.9 | 0.0782 | 0.1080         | 0.0264*                | 0.4420               |
| Serum blood sugar, (mg/dL) | 73.0 ± 20.2 | 117.0 ± 15.9 | 125 ± 19.9 | 0.0041* | 0.0038*        | 0.0053*                | 0.5684               |

* significance. All values are presented as mean ± SEM in each group. ISP indicates isoproterenol; and Lin, linagliptin.
Figure 2. Serum troponin-I levels. Serum troponin-I level in acute phase was higher in the ISP group than the ISP + Lin and the sham groups. However, in the sub-acute phase, troponin-I level was not different among the groups. See text for details.

Figure 3. Serum DPP-4 activity. The serum DPP-4 activity was suppressed in the ISP + Lin group in comparison with the other groups. See text for details.

Figure 4. Histological evaluation. A and B: Representative examples of ventricular tissue in HE and Azan staining. The ventricular tissue injury was induced by ISP injection, which was characterized by cardiomyocyte necrosis and reparative fibrosis especially in the endocardial area. The degree of such injury was suppressed by additional treatment with Lin. The degree of myocardial fibrosis was smaller in the ISP + Lin group than the ISP group (C). See text for details.

The serum free blood sugar was higher in ISP and ISP + Lin treated groups than in sham group (ISP: 117.0 ± 15.97 g/dL versus ISP + Lin: 125.0 ± 19.9 g/dL versus sham: 73.0 ± 20.2 g/dL, *P < 0.05; Table). Histology and myocardial fibrosis: Figure 4A and B show representative examples of ventricular tissues stained by H.E. and Azan staining in the three groups. ISP treated rats, i.e., the ISP and the ISP + Lin groups showed myocardial injury, mononuclear cell infiltration, and reparative fibrosis, especially in the endocardial area, but the degree of myocardial injury seemed relatively lower in the ISP + Lin group in comparison with the ISP group. Figure 4C shows the quantitative comparison of the area of myocardial fibrosis. The area of myocardial fibrosis was smaller in the ISP + Lin group than ISP group. Electrophysiological study: Figure 5 shows the result of electrophysiological parameter. Representative electrophraphs shown in Figure 5A, VERP, MAPD20 and MAPD90 with BCL140 were markedly prolonged in the ISP group in comparison with the sham group (MAPD20: 14 ± 6 versus 11 ± 3 ms, MAPD90: 57 ± 8 versus 44 ± 7 ms, VERP: 74 ± 22 versus 38 ± 10 ms: *P < 0.05), which were considered as the result of ISP induced myocardial injury. In contrast in the ISP + Lin group, such prolongation was relatively suppressed, and the parameters were shorter than the ISP group (MAPD20: 9 ± 2 versus 14 ±
Figure 5. The electrophysiological evaluation. A: Representative examples of electrocardiogram among each group. B: VERP was markedly prolonged in the ISP group in comparison with the sham group. In contrast in the ISP + Lin group, such prolongation was relatively suppressed and VERP was shorter than the ISP group. See text for details. C: Both MAPD20 and MAPD90 were markedly prolonged in the ISP group in comparison with the sham group. In contrast in the ISP + Lin group, such prolongation was suppressed and VERP was significantly shorter than the ISP group. See text for details.

6 ms, MAPD90: 35 ± 6 versus 57 ± 8 ms, VERP: 52 ± 13 versus 74 ± 22 ms; \( P < 0.05 \) (Figure 5B, C). Although the VERP with BCL 140 msec in the ISP + Lin group was still longer than the sham group (VERP: 52 ± 13 versus 38 ± 10 ms; \( P < 0.05 \)), the other parameters in the ISP + Lin group did not show significant difference from the sham group.

VERP, MAPD20 and MAPD90 with BCL120 were markedly prolonged in the ISP group in comparison with the sham group (MAPD20: 14 ± 5 versus 11 ± 2 ms, MAPD90: 56 ± 7 versus 44 ± 6 ms, VERP: 70 ± 21 versus 39 ± 11 ms; \( P < 0.05 \)). In contrast in the ISP + Lin group, such prolongation was relatively suppressed and the parameters were shorter than the ISP group (MAPD20: 7 ± 2 versus 14 ± 5 ms, MAPD90: 31 ± 8 versus 56 ± 7 ms, VERP: 53 ± 15 versus 70 ± 21 ms; \( P < 0.05 \)) (Figure 5B, C).

Evaluation of the oxidative stress: Figure 6A shows the serum d-ROMs levels in the three groups including acute phase data in the ISP and the ISP + Lin groups. The d-ROMs level was higher in the ISP group than in the sham group in the acute phase. Although the ISP + Lin group exhibited higher d-ROMs level than the sham group in the acute phase, it was lower than the ISP + Lin group (sham: 214 ± 55 U.CARR versus ISP [acute]: 404 ± 45 U.CARR versus ISP + Lin [acute]: 337 ± 20 U.CARR, \( P < 0.05 \)). In contrast in the sub-acute phase, the d-ROMs levels did not differ among the groups (sham: 214 ± 55 U.CARR versus ISP: 255 ± 55 U.CARR versus ISP + Lin: 281 ± 41 U.CARR, \( P < 0.05 \)).

In the present study, we have documented the ventricular electrical and structural remodeling in ISP-induced myocardial injury model rats, which can be characterized by endocardium dominant myocardial injury, VERP and MAPD prolongation and marked increase in the oxidative stress especially in its acute phase. Second, we have documented suppressive effect of linagliptin, a DPP-4 inhibitor, on such ventricular structural and electrical remodeling in
this model. This suppression seemed to be parallel to the suppression of the oxidative stress at least in the ventricular tissue.

Characteristics of the ventricular remodeling in the ISP induced myocardial injury: The ISP induced myocardial injury model in rat was originally described by Rona, et al., and it has been used as a model of acute myocardial infarction because it causes endocardium dominant myocardial injury.16) The ISP injection caused ventricular tissue damage like acute myocardial infarction in human. The triggers of such change may possibly be hyperoxidative stress and/or hyper-wall stress which appear during the effect of ISP as a non-selective β-agonist, followed by the release of enormous cytotoxic free radical through the isoproterenol auto-oxidative process. The free radical reacts with proteins, lipid, and nucleic acids and alters lipid peroxidation permeability, then causes integrity breakage of biological membrane, resulting in irreversible damage of the myocardial membrane.17) In this experimental model, the mechanism of heart injury involves multiple factors as mention above, so that it is rationally impossible to set up the positive control group.

In the present study, we controlled the dose of ISP as 70 mg/kg to cause reproducible myocardial injury in Sprague-Dawley rats in accordance with our previous study.18) To set up the dose of isoproterenol, we have performed series of pilot studies and chose 70 mg/kg to achieve significant heart injury and acceptable survival rate.18) Even though our previous study has used 85 or 100 mg/kg of isoproterenol in larger rats, the same dose of ISP caused 60% mortality in our study setting. In this model, endocardial area was predominantly injured with mononuclear cell infiltration and myocardial fibrosis.17) Additionally, we evaluated the changes in the electrophysiological properties in the injured ventricle. The injured ventricle exhibited marked prolongation in the VERP and MAPD, whose change was considered as the electrical remodeling in this model. VERP was longer than MAPD 90 according to Figure 5B and 5C. We understand that MAP duration is an important factor to determine ERP, but the other factors, such as postrepolarization refractoriness and/or activation in partial repolarization, may also affect the ERP, so that MAP and ERP may not be always parallel. To the best of our knowledge, this is the first systematic documentation of the ventricular electrical remodeling in the ISP-induced myocardial injury model. Although these electrophysiological changes did not result in any spontaneous arrhythmia in this model, the abnormal prolongation of the action potential might be considered to construct arrhythmogenic substrate.12,13,19) These changes are also concordant with the previous reports regarding rat acute myocarditis model, therefore, the downregulation of some ion channels may possibly be expected.12,13)

Suppressive effect of linagliptin on the ventricular remodeling: In this study, we also performed ISP injection to the rats under linagliptin treatment to test the suppressive effect of linagliptin on the ISP inducing changes in this rat model. Although linagliptin, one of the DPP-4 inhibitors, has been used as an incretin-based anti-diabetic medicine, its cardioprotective effect has been suggested in several recent studies.20) In our present study, the ISP in-

Figure 6. Serum d-ROMs levels and DHE staining. A: Serum d-ROMs level in the acute phase was higher in the ISP group than the ISP + Lin and the sham groups. In contrast in the sub-acute phase, d-ROMs level was not different among the groups. See text for details. B: Representative examples of DHE staining. DHE was clearly stained in the nuclei of the myocardial cells. Green pigmented nuclei indicate increased oxidative stress, and they were observed in the ISP and ISP + Lin groups. The degree of the DHE staining was more prominent in the ISP group than the ISP + Lin group. The specificity of DHE signals for O₂• was confirmed by pre-incubation with their inhibitor, polyethylene glycol-superoxide dismutase (PEG-SOD). See text for details.
duced changes, i.e., myocardial tissue injury, mononuclear cellular infiltration, fibrosis, VERP prolongation, MAPD prolongation, and increased oxidative stress, were all attenuated by linagliptin treatment. This indicates the cardioprotective effect of linagliptin at least in a specific experimental condition in our study.

Mechanism of suppression of ventricular tissue damage by linagliptin: The mechanism of suppressive effect of linagliptin on the ISP induced tissue injury is unclear, but at least partly explained by suppression of the hyper-oxidative stress. ISP is a strong inducer of oxidative stress44 which was also confirmed in our present study by serum d-ROMs level in the acute phase and DHE staining in the sub-acute phase. The linagliptin treatment attenuated such increases in oxidative stress which was parallel to the attenuation of ventricular structural and electrical changes. DPP-4 is a ligand for membrane-bound adenosine deaminase (ADA) which scavenges adenosine from extracellular environment.40 In myocardial infarction rat model, ADA binds to the cellular membrane by DPP-4,21 causing energy depletion by degrading adenosine. DPP-4 inhibitor suppresses binding of DPP-4 to ADA, then result in maintenance of adenosine level and decrease of inosine level. Given that adenosine suppresses superoxide production by signaling through adenosine A1 receptors, it suppresses the appearance of hyperoxidative state. On the other hand, decrease of inosine level suppresses superoxide production by decreasing the formation of xanthine oxidase substrates.50 Furthermore, DPP-4 inhibitor inhibits the degradation of GLP-1 by suppressing plasma DPP-4 enzyme. Increased GLP-1 facilitates adenylyl cyclase activity and causes increase of cyclic adenosine monophosphate (cAMP). Finally, cAMP facilitates protein kinase A (PKA) activity and upregulates heme oxygenase-1 expression (HO-1). HO-1 is one of the stress-induced proteins and protects various cells against the oxidative stress.22,23 This HO-1 upregulation may also express as attenuation of tissue injury caused by hyperoxidative stress.46 The previous study reported that DPP-4 inhibitor have antioxidative effect via a cAMP/PKA dependent pathway in non-diabetic infarcted rats.20 In this study, such effect of DPP-4 inhibitor is highly speculated, since ISP would enhance PKA activity.

Mechanism of suppression of electrical remodeling by linagliptin: If the electrical changes, i.e., prolongation of VERP and MAPD, are the results of tissue mechanical injury, suppressive effect of linagliptin on the electrical changes could be explained by the suppression of tissue injury itself. However, these electrical changes are concordant with the changes observed in the previous reports regarding heart failure models.25,26 Additionally, we have previously reported that primary oxidative stress can induce VERP and MAPD prolongation by reducing the expression levels of erg and SERCA2A in a model of hyperoxidative stress rats.17 Therefore, these electrical changes observed in the ISP induced myocardial injury model might also appear through the influence of the hyperoxidative stress. In this study, the ISP induced ventricular arrhythmias were not evaluated. Cellular calcium dysregulation due to oxidative stress may provoke calcium-triggered arrhythmias. In this story, suppressive effect of linagliptin may also be explained by antioxidative effect of linagliptin as described above, i.e., the suppressor of PKA/HO-1 pathway signaling. Although Liu, et al. has reported different mechanism of ISP induced MAPD prolongation,27 i.e., downregulation of SERCA2 and RyR2, and upregulation of Cav1.2 protein expression. They also emphasized the effect of hyperoxidative stress as the mechanism, which does not contradict to our postulated mechanism of linagliptin as the suppressor of the oxidative stress.

Ventricular arrhythmias are known to be one of the important problems after myocardial infarction.28,29 This study suggests that linagliptin, a DPP-4 inhibitor, may attenuate structural and electrical remodeling in injured heart, resulting in suppression of injury area and subsequent arrhythmia. Further clinical data are necessary to free effect of DPP-4 inhibitor on ventricular arrhythmia. Study limitations: There are several limitations. First, the electrophysiological characteristics of myocardium cells were not evaluated in the patch-clamp method. We did not evaluate the changes in ion channel expressions as the mechanism of VERP and MAPD prolongation. Second, the precise mechanism of antioxidative effect of linagliptin was not clarified from story, which points should be clarified in future studies in specific designs. We also think evaluation of antioxidative stress effects should be important next steps of this study. Three, present study was not accessed using diabetes mellitus model rat. Four, present study was not accessed using different dosing and/ or use of other DPP-4 inhibitors and linagliptin alone. Such assessment may lead to conclude documented results were class effect of DPP-4 inhibitor or just specific effect of linagliptin. Finally, Serum free blood sugar levels were higher in Isoproterenol groups and Isoproterenol + linagliptin group than sham group. Such phenomenon was caused by isoproterenol effect. However, Serum free blood sugar levels were higher in Isoproterenol + linagliptin group than Isoproterenol group. We cannot explain this phenomenon, but the effect of linagliptin may be weak in individual with normal blood sugar level. Further clinical data are necessary to clarify the effect of DPP-4 inhibitor on ventricular arrhythmia.

Conclusion

In this ISP-induced myocardial injury rat model, an electrical remodeling characterized by VERP and MAPD prolongation was observed along with the structural remodeling (i.e., endocardium dominant myocardial injury). The hyperoxidative stress was considered to play a role in causing such changes. Linagliptin, a DPP-4 inhibitor, suppressed such structural and electrical changes possibly through the antioxidative effect.

Disclosures

Conflicts of interest: The compound of linagliptin was offered from Boehringer-Ingelheim GmbH, Germany, but no financial support was offered. The authors state that there were no conflicts of interest.
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