Effect of gemigliptin on cardiac ischemia/reperfusion and spontaneous hypertensive rat models

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ABSTRACT Diabetes is associated with an increased risk of cardiovascular complications. Dipeptidyl peptidase-4 (DPP-IV) inhibitors are used clinically to reduce high blood glucose levels as an antidiabetic agent. However, the effect of the DPP-IV inhibitor gemigliptin on ischemia/reperfusion (I/R)-induced myocardial injury and hypertension is unknown. In this study, we assessed the effects and mechanisms of gemigliptin in rat models of myocardial I/R injury and spontaneous hypertension. Gemigliptin (20 and 100 mg/kg/d) or vehicle was administered intragastrically to Sprague–Dawley rats for 4 weeks before induction of I/R injury. Gemigliptin exerted a preventive effect on I/R injury by improving hemodynamic function and reducing infarct size compared to the vehicle control group. Moreover, administration of gemigliptin (0.03% and 0.15%) powder in food for 4 weeks reversed hypertrophy and improved diastolic function in spontaneously hypertensive rats. We report here a novel effect of the gemigliptin on I/R injury and hypertension.
the Republic of Korea and the global market. However, the effects of gemigliptin on animal models of CVD have not been reported. We assessed the effects of gemigliptin on rats with ischemia/reperfusion (I/R) injury and spontaneous hypertension.

**METHODS**

**Materials**

Gemigliptin tartrate sesquihydrate (LG Chemical, Daejeon, Korea), 2,3,5-triphenyl tetrazolium chloride (TTC; Sigma-Aldrich, St. Louis, MO, USA), isoflurane (Primah Healthcare, Andhra Pradesh, India), and Rompun (xylazine; Bayer, Leverkusen, Germany) were used in this study.

**Animals**

Male Sprague-Dawley (n = 7–9; 4 weeks old; 180–200 g), male Wistar-Kyoto (WKY, n = 6; 8 weeks old; 270–310 g) rat and male spontaneously hypertensive rats (SHR, n = 8; 8 weeks old; 270–310 g) were obtained from a commercial breeder (Orient Bio Inc., Seongnam, Korea). All animals were housed with *ad libitum* access to water and food under a 12/12 h light/dark cycle at 22 ± 2°C. The rats were allowed to acclimatize for one week prior to the experiment. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Korea Institute of Toxicology (IACUC approval No. 1704-0137).

**Surgical preparation**

I/R injury was induced in the rats by ligation of the left anterior descending (LAD) coronary artery for 10 min followed by reperfusion for 24 h [14]. Briefly, anesthesia was induced using a mixture of isoflurane (3 ml/min) and 95% O₂ and 5% CO₂ in an induction chamber. Rats were then sedated by intraperitoneal injection of Rompun (1 mg/kg). The trachea was intubated with a cannula connected to a Harvard rodent ventilator (Model 683; Harvard Apparatus, Holliston, MA, USA) for artificial respiration. The heart was exposed by performing a left thoracotomy, and the LAD was ligated 2–3 mm from its origin and loosened. During the period of ischemia, the body temperature of the rats was maintained by placing them on a 37°C heating pad (L.M.S. Korea, Seongnam, Korea). The rats were randomly divided (n =

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**Fig. 1. Effect of gemigliptin (GG) on ischemia/reperfusion (I/R)-induced infarct size.** (A) Change in rat weight as a function of the period of treatment. (B) Representative illustrations of heart sections stained with 2,3,5-triphenyl tetrazolium chloride (TTC). The infarcted area is shown in white color. The white dotted lines indicate the infarcted areas. Six sections cut into 2 mm transverse of the heart to visualize the infarcted areas. (C) Infarct size is quantified as a percentage of total slice area. All data are shown as mean ± standard error of the mean. N = 7–9 hearts per group. Sham vs. I/R, I/R+GG 20, I/R+GG 100 (**p < 0.01; ***p < 0.001), I/R vs. I/R+GG 20, I/R+GG 100 (##p < 0.01; ###p < 0.001).
7–9) into the (1) sham group (underwent surgery but not I/R); (2) I/R group (induction of I/R); and (3, 4) gemigliptin + I/R groups (administered 20 or 100 mg/kg gemigliptin in distilled water intragastrically for 4 weeks before induction of I/R).

Hypertensive rat model and treatment regimen

Male WKY and SHR rats were randomly divided into four equal-sized groups: (1) WKY group, normotensive WKY rats; (2) hypertension group, SHR rats; and (3, 4) gemigliptin + hypertension groups, SHR rats fed gemigliptin powder (0.03% and 0.15%) for four weeks.

Drug administration

Gemigliptin tartrate sesquihydrate powder was suspended in distilled water and administered daily to rats at 20 or 100 mg/kg of body weight by oral gavage. Also, gemigliptin powder was mixed into the rats food in the gemigliptin + hypertension groups.

Hemodynamic measurements

Left ventricle (LV) function was evaluated using a pressure-volume (P-V) conductance catheter (ADV500 Admittance Pressure Volume Control Unit; Transonic Scisense Inc., London, ON, Canada). The catheter (1.9 F; Transonic Scisense Inc.) was inserted into the right carotid artery and advanced into the LV chamber to record the LV P-V relationship. We evaluated the following hemodynamic parameters: heart rate (HR), end systolic pressure (ESP), end diastolic pressure (EDP), dP/dt maximum (dP/dt max), dP/dt minimum (dP/dt min), end systolic volume (ESV), end diastolic volume (EDV), stroke volume (SV), cardiac output (CO), ejection fraction (EF), and stroke work (SW).

Myocardial infarct analysis

TTC (Sigma-Aldrich) staining was performed to evaluate myocardial infarct size. After reperfusion, the hearts were isolated, washed in phosphate-buffered saline (PBS) and sectioned into 2 mm transverse slices. After incubation in 1% TTC at 37°C in PBS for 15 min, the heart slices were imaged. Ischemic areas (red) and infarct areas (white) were determined. The ratio of infarcted myocardium to total myocardial tissue was calculated using the infarct area / total heart area (%). Infarct sizes were measured using Image J software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Results are expressed as means ± standard error of the mean. Data were subjected to one-way analysis of variance with Tukey’s post-hoc test using Prism 5.01 (GraphPad Software Inc., La Jolla, CA, USA). Values of p < 0.05, 0.01 and 0.001 were considered to indicate significant and highly significant differences, respectively.

RESULTS

Gemigliptin reduces myocardial damage following I/R injury

Body weight did not differ significantly among the four groups at days 1, 2, 3 and 4 (Fig. 1A). Therefore, it seemed gemigliptin was not toxic to the rats.

To examine the cardioprotective effects of gemigliptin, the size of the infarcted myocardium was determined through TTC staining. TTC stains healthy viable myocardium deep-red color, while pale white color as necrosis myocardium. Myocardial in-
farcts in the I/R group were significantly larger than those in the sham group and were reduced by treatment with 20 and 100 mg/kg gemigliptin (Fig. 1B, C). These results demonstrated that gemigliptin had a protective effect against I/R-induced myocardial damage.

**Gemigliptin improves the recovery of cardiac blood pressure**

HR and EDP did not differ among the groups (Fig. 2A, C). Compared with the I/R group, the I/R + gemigliptin 20 and 100 mg/kg groups showed significantly elevated ESP, dP/dt max, and dP/dt min values (Fig. 2B, D, E). These data suggested that gemigliptin improved against I/R-induced decline in cardiac contractile function at the dosages tested.

**Gemigliptin improves functional recovery following I/R injury**

We next investigated the functional mechanism underlying the protective effect of gemigliptin on I/R-induced cardiomyocyte injury. The ESV and EDV were significantly higher while the SV, CO, EF, and SW were significantly lower in the I/R group compared with the sham group. However, all these values were improved by gemigliptin (Fig. 3A–F). The I/R group had a significantly lower systolic LV pressure than that of the gemigliptin groups (Fig. 3G). Furthermore, the loops of rats in the I/R group showed a prominent rightward displacement along the LV volume axis, suggesting cardiac injury. Therefore, gemigliptin increased the LV contraction and relaxation rates after I/R.

**Effect of gemigliptin on CV parameters on SHR model**

I/R injury and hypertension induce LV remodeling and alter LV function. Gemigliptin exerted significant effects on the ESP, dP/dt max, dP/dt min, ESV, and EF values, but not on HR (Fig. 4A–F). The slope of the end-diastolic P-V relationship was steeper in SHRs than WKY rats (Fig. 4G) and was decreased by gemigliptin. Therefore, gemigliptin reduced LV hypertrophy and improved LV compliance.

**DISCUSSION**

In the present study, we report here for the first time that gemigliptin exerts a protective and preventive effect on I/R injury and hypertension in a dose-dependent manner, respectively. We show that gemigliptin prevented I/R injury by improving the EF, dP/dt min, dP/dt max, ESP, and infarct size and decreasing the ESV and EDV, but did not affect HR. Also, gemigliptin treatment in SHRs showed a significant reduction in blood pressure without changes in heart rate. Therefore, long-term administration of gemigliptin may exert a cardioprotective and preventive effect.

Gemigliptin is an oral diabetic agent for the treatment of type 2 diabetes by inhibiting DPP-IV [15]. Administration of 20 or
Effect of gemigliptin in rats

100 mg/kg of gemigliptin by oral gavage results in an increase in the GLP-1 level [16]. Following the administration of a single oral dose of gemigliptin (20 or 100 mg/kg) to healthy subjects, its mean plasma concentration was approximately 5- to 6-fold and 27- to 30-fold, respectively, of the clinical dose of 50 mg [17]. In this study, gemigliptin increased GLP-1 activation in a dose-dependent manner, and the plasma concentration was higher than the clinical dose used in humans (data not shown). Several mechanisms for the improvement of cardiac function by DPP-IV inhibitors have been suggested. Sitagliptin and vildagliptin exert a cardioprotective effect by increasing the expression of GLP-1 in a glucose-dependent manner [18]. Saxagliptin, via its antioxidant activity, exert a cardioprotective effect against I/R injury in diabetic and normoglycemic rats [19]. Other substrates of DPP-IV, such as chemokines, neuropeptides, and stromal cell-derived factor-1, may contribute to the protective effects of DPP-IV inhibitors against I/R injury [20].

The ESP was significantly higher in SH rats and was reduced by gemigliptin. We measured the plasma concentration of DPP-IV after analyzing cardiac function, and gemigliptin-dose-dependent effects on the concentrations of DPP-IV were observed between 0.03% and 0.15% of gemigliptin (data not shown). Gemigliptin 0.03% and 0.15% is equivalent to 20 and 100 mg/kg/day, respectively, based on daily food intake. However, plasma concentrations of DPP-IV were higher in the I/R rats than in the SH rats (data not shown). DPP-IV inhibitors reduce blood pressure mainly by improving endothelial function [21] and possibly by regulating cardiac calcium levels and glucose transporter 4 translocation [21-23].

This study had several limitations. We did not determine the mechanism of the CV effects of gemigliptin; however, the CV effects were likely independent of its glucose-lowering effect because the models used did not involve high glucose levels. Further studies should examine the mechanisms underlying the above-mentioned effects of gemigliptin.

In summary, gemigliptin exerted a cardioprotective and preventive effect by reducing the size of cardiac infarcts and lowering blood pressure. Therefore, gemigliptin exerts a beneficial effect on blood glucose and the CV system in the presence of cardiac I/R injury.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.
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