Exendin-4 inhibits atrial arrhythmogenesis in a model of myocardial infarction-induced heart failure via the GLP-1 receptor signaling pathway

JINGJING CHEN[1], SHUNEN XU[2], LONG WANG[1], WEI ZHOU[1], PING LI[1], NA DENG[1], QIAN TANG[1], YONGKANG LI[1], LIRONG WU[1], JIULIN CHEN[3] and WEI LI[1]

Departments of [1]Cardiology and [2]Orthopedic Surgery, The Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou 550004; [3]Department of Cardiology, Qian Xi Nan People's Hospital, Bijie, Guizhou 562400, P.R. China

Received March 19, 2020; Accepted July 1, 2020

DOI: 10.3892/etm.2020.9089

Abstract. Glucagon-like peptide-1 receptor (GLP-1 receptor) agonists are considered to exert cardioprotective effects in models of acute and chronic heart disease. The present study aimed to investigate the role of exendin-4 (a GLP-1 receptor agonist) in atrial arrhythmogenesis in a model of myocardial infarction (MI)-induced heart failure and to elucidate the mechanisms underlying its effects. For this purpose, male Sprague-Dawley rats underwent sham surgery or left anterior descending artery ligation prior to being treated with saline/exendin-4/exendin-4 plus exendin9-39 (an antagonist of GLP-1 receptor) for 4 weeks. The effects of exendin-4 on atrial electrophysiology, atrial fibrosis and PI3K/AKT signaling were assessed. Rats with MI exhibited depressed left ventricular function, an enlarged left atrium volume, prolonged action potential duration, elevated atrial tachyarrhythmia inducibility, decreased conduction velocity and an increased total activation time, as well as total activation time dispersion and atrial fibrosis. However, these abnormalities were attenuated by treatment with the GLP-1 receptor agonist, exendin-4. Moreover, the expression levels of collagen I, collagen III, transforming growth factor-β1, phosphorylated PI3K and AKT levels in atrial tissues were upregulated in rats with MI. These changes were also attenuated by exendin-4. It was also found that these exedin-4-mediated attenutations were mitigated by the co-administration of exendin9-39 with exendin-4. Overall, the findings of the present study suggested that exendin-4 decreases susceptibility to atrial arrhythmogenesis, improves conduction properties and exerts antifibrotic effects via the GLP-1 receptor signaling pathway. These findings provide evidence for the potential use of GLP-1R in the treatment of atrial fibrillation.

Correspondence to: Dr Wei Li, Department of Cardiology, The Affiliated Hospital of Guizhou Medical University, 28 Guiyi Street, Guiyang, Guizhou 550004, P.R. China
E-mail: liwei249188@163.com

Key words: cardiac electrophysiology, fibrosis, heart failure

Introduction

Heart failure is the leading cause of heart disease-associated death worldwide and is caused by various pathologies, including myocardial infarction and hypertension (1). Heart failure is one of the most common causes of atrial fibrillation (AF), which induces atrial electrical and structural remodeling, as well as increased atrial fibrosis (2). Atrial fibrosis contributes to the development and persistence of AF and thus plays a role in the AF-induced impairment of electrical conduction between cardiomyocytes, leading to reduced conduction velocity and increased conduction dispersion (3).

Glucagon-like peptide-1 (GLP-1), an incretin released postprandially, has been shown to exert cardioprotective effects in addition to glycemic regulatory effects (4). Following its release, GLP-1 is quickly degraded by dipeptidyl peptidase 4 (DPP-4). GLP-1 receptor activation preserves cardiac performance by activating multiple pro-survival kinases and modulating cell apoptosis and energy utilization (5-7), and exendin-4 (a long-acting GLP-1 analogue)-mediated GLP-1 receptor activation in models of MI inhibits cardiac remodeling by mitigating oxidative stress-induced injury (6) and attenuating interstitial fibrosis (4). GLP-1 receptors are expressed in atrial myocytes and arteries (8). Previous studies have focused mainly on ventricular structural remodeling (6,9). Thus, whether exendin-4 influences atrial arrhythmogenesis in a rat heart failure model remains unknown.

The acute activation of the AKT signaling pathway, a survival signaling pathway, protects the heart from ischemia/reperfusion injury (10). In contrast, the long-term activation of the AKT signaling pathway leads to cardiac hypertrophy and fibrosis (11). A previous study demonstrated that GLP-1 receptor activation protected rat hearts from ischemia/reperfusion injury via the activation of the AKT pathway (12). To better understand the association between the GLP-1 receptor and atrial fibrosis in heart failure, the present study aimed to evaluate the effects of exendin-4 treatment on atrial fibrosis and arrhythmia vulnerability in a rat model of MI-induced heart failure and to investigate the mechanisms underlying these effects.

Materials and methods

Experimental animals. The experiments were performed in strict accordance with the recommendation in the Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory Animal Research, National Research Council, Washington, D.C., National Academy Press, 2011 (13). Moreover, all experimental protocols were approved by the Experimental Animal Care and Institutional Animal Ethical Committee of Guizhou Medical University (Guiyang, China).

In total, 88 male Sprague-Dawley rats weighting between 200-250 g, were purchased from Hunan Silaike Jingda Laboratory Animal Co., Ltd. The rats were acclimatized at 25˚C with a 12-h light/dark cycle and relative humidity of between 40 and 70%, and had free access to food and water.

The rats were randomly divided into the following four groups: i) A sham-operated group comprising of healthy rats without heart failure that received 4 weeks of intraperitoneal saline treatment; ii) a myocardial infarction group (MI group) comprising of rats with coronary ligation-induced heart failure that received 4 weeks of intraperitoneal saline treatment; iii) an MI plus Exendin-4 group (MI+Ex-4 group) comprising of rats with heart failure that received an intraperitoneal injection of 10 μg/kg/day (14) exendin-4 (the dose was selected based on the results of a previous study by the authors) (9) for 4 weeks and iv) an MI plus exendin9-39 and exendin-4 group (MI+Ex-4+Ex9-39 group) comprising of rats with heart failure that received an intraperitoneal injection of 150 μg/kg/day (15) exendin9-39 and 10 μg/kg/day exendin-4 for 4 weeks. Each group comprised 22 rats. The drugs and saline were administered beginning at 24 h after surgery, and 1/2 of the total dose was administered every 12 h. The experimental groups and treatment schedules are presented in Fig. 1.

Coronary ligation. The coronary ligation procedure performed herein was described in a previous study by the authors (16). The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg, Sigma-Aldrich; Merck KGaA). After full anesthesia was achieved when pain reflex disappeared, artificial respiration was initiated using a volume-constant rodent ventilator. The chest was opened through a left thoracotomy at the third intercostal space, after which left anterior descending (LAD) artery ligation was performed using a 7-0 silk suture. The rats in the sham-operated group underwent the same surgical procedure, but did not undergo coronary artery ligation.

Echocardiographic measurements. Echocardiography (Vevo 770; Visual Sonics) was performed under anesthesia with an intraperitoneal injection of pentobarbital sodium (40 mg/kg, Sigma-Aldrich; Merck KGaA) to assess the left ventricular function 4 weeks after the administration of saline/Ex-4/Ex-4+Ex9-39. Atrial analyses included left atrial (LA) dimension at end diastole and systole and LA fractional shortening. A pulse-wave Doppler was used to assess mitral valve flow as an index of diastolic function.

Hemodynamic measurements. Systemic hemodynamic analyses were measured with 2.0 F Millar catheters inserted into the right carotid artery and then advanced into the left ventricle in rats anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg, Sigma-Aldrich; Merck KGaA). Following the hemodynamic measurements, the rats were euthanized by cervical dislocation under the aforementioned anesthesia. The lungs and tibiae of the rats were dissected and measured for the lung weight/tibia length (LW/TL, mg/mm) ratio calculation.

Preparation of Langendorff-perfused hearts. The rats (n=12 in each group) were anesthetized as aforementioned, heparinized (heparin sodium, 400 U, i.p., Sigma-Aldrich; Merck KGaA). Their hearts were rapidly harvested, rinsed and perfused in a Langendorff perfusion system (ADInstruments) with oxygenated HEPES-buffered Tyrode solution containing the following components (mmol/l): NaCl, 135; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1; Na_2HPO4, 0.3; HEPES, 10 and glucose, 10 (pH 7.3) at a constant pressure (70-90 cm H_2O) and temperature (37.0±0.5˚C). The hearts were perfused for 20 min prior to microelectrode array recording, monophasic action potential (MAP) recordings and atrial tachyarrhythmia susceptibility.

Microelectrode array (MEA) recording. MEA recording was conducted on the Langendorff-perfused hearts for extracellular electrophysiological analysis and excitation propagation observations, which were performed 4 weeks after MI. An array of 32 monopolar electrodes (32 Map) capable of covering a 3x3-mm square (inter-electrode distance of 500 μm) was placed on the left atrial appendage (LAA). The 32 Map signals were obtained and processed using Cardio 2D_2.6.2 and Cardio 2D+_2.4.2 software (Multi Channel Systems MCS GmbH). The total activation time (TAT) was defined as the longest possible period in which the excitation was passed from the first excited electrode to the last excited electrode. The TAT difference of each heartbeat was measured for the TAT dispersion as an index of conduction homogeneity. TAT dispersion was estimated by the standard deviation of the total activation time in the mapped area in 60 sec. Conduction velocity was assessed by linear regression analysis of the isochrones distance vs. activation time in MEA recording (17).

MAP recordings and atrial tachyarrhythmia susceptibility. A pair of platinum stimulating electrodes were placed on the epicardial surface of the right atrial appendage for programmed stimulation, and custom-made-Ag-AgCl electrodes consisting of two 0.25-mm Teflon-coated silver wires were positioned on the LAA for MAP recording. The 20, 50 and 90 action potential durations (APD_{20}, APD_{50} and APD_{90}) were calculated at a pacing cycle length (PCL) of 250 msec by performing at least eight successive MAPs. Burst pacing (2-msec pulse width at 20 Hz, 2-sec burst duration) was used to induce atrial tachyarrhythmias, which were defined as continuous atrial contractions lasting >2 sec. Sustained atrial tachyarrhythmia was defined as contractions lasting >30 sec.

Histological analysis. The rats were euthanized by cervical dislocation under anesthesia (intraperitoneal pentobarbital sodium, 40 mg/kg, Sigma-Aldrich; Merck KGaA) following the administration of the drugs for 4 weeks. The hearts were excised and arrested in diastole with 10% KCl, fixed in 10%

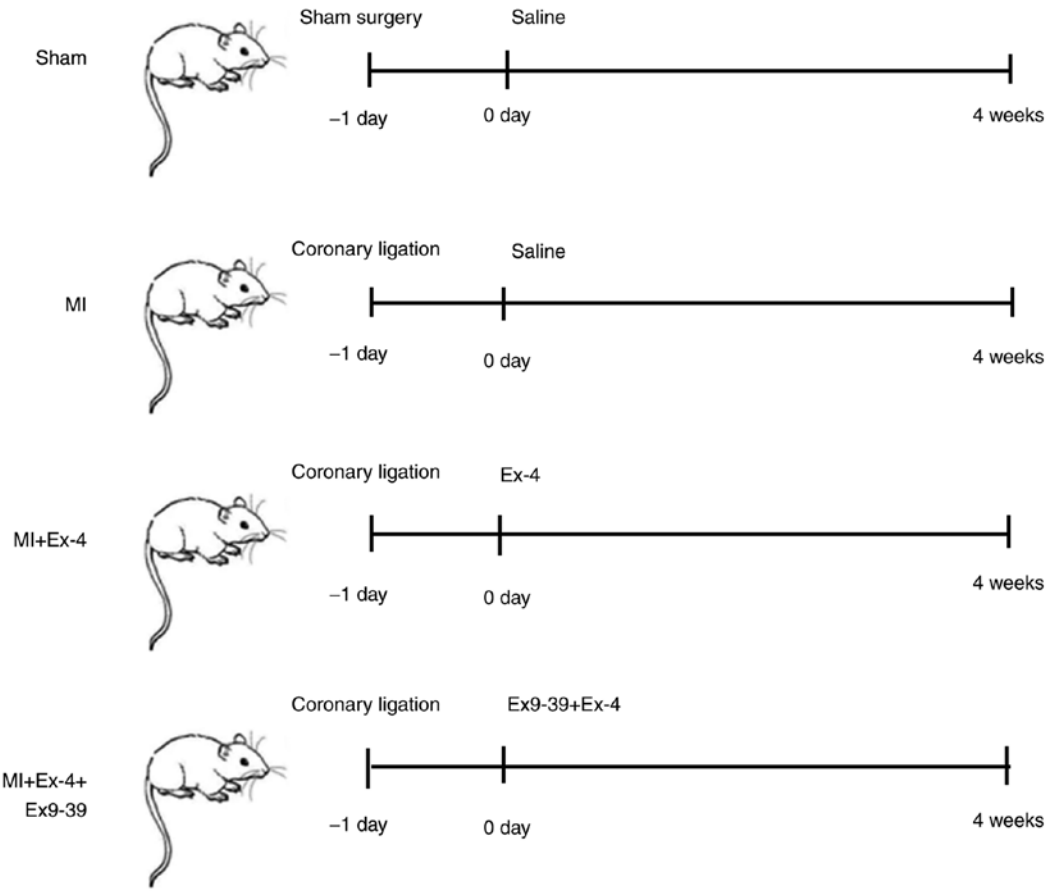

Figure 1. Experimental groups and treatment schedules. MI, myocardial infarction; Ex-4, exendin-4; Ex9-39, exendin9-39.

buffered formalin at room temperature for 24 h and embedded in paraffin. The tissues were subsequently cut into 3-μm thick sections and stained with Masson's trichrome at room temperature for 2 h to assess interstitial collagen deposition. All heart sections were examined, and images were captured via light microscopy (magnification, x100 and x400; BX-50; Olympus Corporation). Data analysis was performed using Image-Pro Plus 6.0 software (Media Cybernetics, Inc.). The collagen volume fraction (CVF) was calculated as a percentage of the interstitial collagen area.

Measurement of plasma GLP-1 and atrial natriuretic peptide (ANP) concentrations. Plasma GLP-1 (n=6; cat. no. EZGLP1T-36K; Sigma-Aldrich; Merck KGaA) and ANP (n=6; cat. no. E-EL-R0017; Elabscience, Inc.) concentrations were assessed using post-caval blood samples using enzyme immunoassay kits.

Western blotting. Total protein was extracted from the rat LAA tissue using RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein concentration was examined using a Bicinchoninic Acid Protein Assay kit (cat. no. AS1086; Wuhan Aspen Biotechnology Co., Ltd.). Protein samples (40 μg/lane) were loaded onto an 8-12% gel, separated using SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were subsequently incubated with primary antibodies against the GLP-1 receptor (cat. no. ab111125; 1:1,000, Abcam), collagen I (Col I; cat. no. ab34710; 1:200; Abcam), collagen III (Col III; cat. no. ab184993; 1:2,000; Abcam), transforming growth factor (TGF)-β1 (cat. no. ab179695; 1:600; Abcam), total AKT (1:1,000, Abcam, ab8805), phosphorylated AKT (cat. no. ab38449; 1:2,000, Abcam), total PI3K (cat. no. ab154598; 1:1,000, Abcam) and phosphorylated PI3K (cat. no. ab191606; 1:200; Abcam) overnight at 4˚C. Following which, membranes were incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-goat immunoglobulin G (IgG) (cat. no. AS1108; 1:10,000; Wuhan Aspen Biotechnology Co., Ltd.) or HRP-conjugated goat anti-rabbit IgG (cat. no. AS1107; 1:10,000; Wuhan Aspen Biotechnology Co., Ltd.) secondary antibodies for 2 h at room temperature. Chemiluminescence reagent (ECL kit; Beyotime Institute of Biotechnology) was used to visualize the proteins, and quantitative densitometric analysis was performed using Image-Pro Plus 6.0 software (Media Cybernetics, Inc.).

Reverse transcription-quantitative PCR (RT-qPCR). RT-qPCR was used to analyze *GLP-1* receptor mRNA expression. Total RNA was isolated from the LAA tissues using a Total RNA Extraction kit (RN0401; Aidlab Biotechnologies Co., Ltd.). RNA samples (4 μg) were reverse transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis kit (cat. no. R101-01; Vazyme Biotech Co., Ltd.). The reverse transcription temperature protocol was as follows: 25˚C for 5 min, 50˚C for 15 min, 85˚C for 5 min and 4˚C for 10 min. qPCR was performed using using SYBR Green master mix (Roche Diagnostics). The following thermocycling conditions were used for the qPCR: 50˚C for 2 min, 95˚C for 10 min; followed by 40 cycles of denaturation

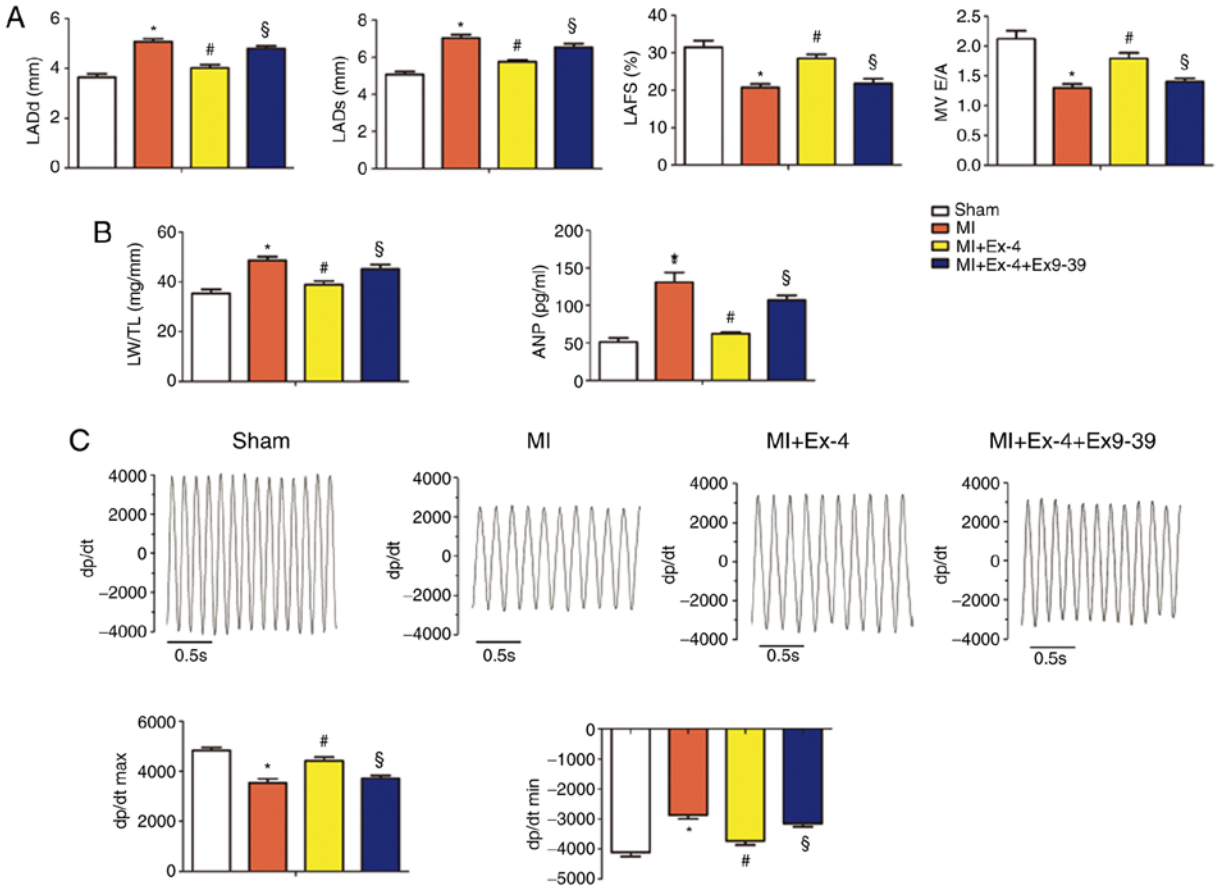

Figure 2. The GLP-1 receptor agonist, exendin-4, preserves heart function and hemodynamics. (A) Echocardiographic data for the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 groups (n=9 animals per group). MV E/A, LADd, LADs and LAFS were analyzed using echocardiography. (B) LW/TL ratios and the plasm levels of ANP in the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 groups (n=9 animals per group). (C) Representative recordings of dp/dt and the statistical analyses of dP/dtmax, dP/dtmin, SBP, DBP, MBP in the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 rat hearts at 4 weeks following sham or MI surgery (n=9 animals per group). *P<0.05 vs. the sham-operated group, #P<0.05 vs. the MI group, §P<0.05 vs. the MI+Ex-4 group. MI, myocardial infarction; Ex-4, exendin-4; Ex9-39, exendin9-39; LW/TL, lung weight to tibia length ratio; MV E/A, mitral valve E/A; LADd, LA dimension in ventricular diastole; LADs, LA dimension in ventricular systole; LAFS, LA fractional shortening; ANP, atrial natriuretic peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure.

at 95˚C for 30 sec, annealing and extension at 60˚C for 30 sec. The data were analyzed using the $2^{-\Delta\Delta Cq}$ method (18). GLP-1 receptor mRNA expression levels were normalized to GAPDH expression levels. The following primers used were used in the present study: GAPDH, forward: 5'-ACAGCAACAGGGTGG TGGAC-3' and reverse: 5'-TTTGAGGGTGCAGCGAACTT-3; and GLP-1 receptor, forward: 5'-TCTTCTGCAACCGAACCT TT-3' and reverse: 5'-TGCCCTTGGAGCACACTACT-3.

Statistical analysis. Statistical analysis was performed using SPSS 22.0 software (IBM Corp). All experiments were independently performed three times. Numerical data are expressed as the means ± standard error of the mean and were analyzed using one-way analysis of variance (ANOVA), followed by the Bonferroni's multiple comparison test. Categorical data, which included ATA incidence and sustained ATA incidence, were analyzed by the χ^2 test and Fisher's exact test. P<0.05 was considered to indicate a statistically significant difference.

Results

Exendin-4 improves heart function following MI. Post-MI left atrium dimensions were increased and atrial fractional shortening decreased compared with sham rats (Fig. 2A). Furthermore, the LW/TL ratios and plasma ANP concentration were increased following MI (Fig. 2B). Hemodynamic measurements revealed reduced contractility (dP/dtmax) and relaxation (dP/dtmin) in the rats with MI compared with sham rats (Fig. 2C). These abnormalities were attenuated by exendin-4 treatment. Moreover, the co-administration of exendin9-39 and exendin-4 diminished the protective effects of exendin-4 on cardiac function.

Exendin-4 ameliorates atrial electrical remodeling in heart failure. To determine whether exendin-4 treatment exerts effects on atrial electrophysiology, the APDs and atrial tachyarrhythmia inducibility were measured in Langendorff-perfused hearts. It was found that the APD_{50} and APD_{90} were prolonged following MI compared with sham rats; however, the APD_{50} and APD_{90} were shorter in the exendin-4 group compared with the MI group (Fig. 3A). Additionally, atrial tachyarrhythmias occurred in two out of 12 sham-operated hearts and 11/12 hearts with MI. However, atrial tachyarrhythmias occurred in five out of 12 hearts treated with exendin-4 (Fig. 3B). Furthermore, the incidence of sustained atrial tachyarrhythmias inducibility was decreased in the hearts treated with exendin-4 compared with the hearts with

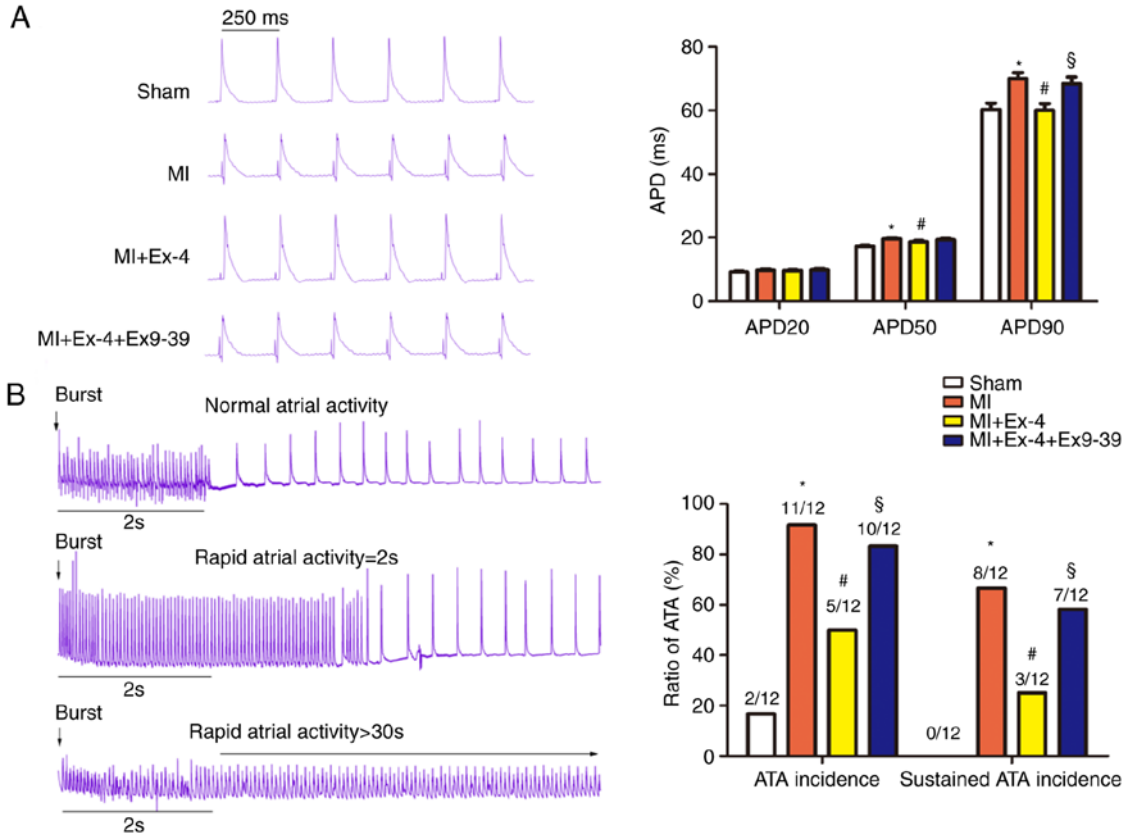

Figure 3. The GLP-1 receptor agonist, exendin-4, improves atrial electrophysiological function properties. (A) Representative recordings of action potentials and quantitative analysis of the APD_{20}, APD_{50} and APD_{90} of LAA in the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 rat hearts at four weeks following sham or MI surgery (n=12 animals per group). (B) Representative recordings of burst pacing-induced atrial tachyarrhythmias in the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 rat hearts at four weeks following sham or MI surgery and statistical analyses of these data (n=12 animals per group). $^{*}P<0.05$ vs. the sham-operated group, $^{\#}P<0.05$ vs. the MI group, $^{\S}P<0.05$ vs. the MI+Ex-4 group. MI, myocardial infarction; Ex-4, exendin-4; Ex9-39, exendin9-39; APD, action potential duration; LAA, left atrial appendage.

MI (Fig. 3B). The effects of exendin-4 on atrial electrophysiology were abolished by the co-administration of exendin9-39.

Exendin-4 modulates disordered excitation transmission. Representative excitation transmission maps of the surface of the LAA in each group are presented in Fig. 4A. In the rats in the sham-operated group, propagation was conducted rapidly from the first excited electrode to the last excited electrode. Following MI, the signal proceeded slowly, a change accompanied by an increased total activation time and a decreased conduction velocity compared with sham rats (Fig. 4C). Exendin-4 treatment increased the conduction velocity and decreased the total activation time compared with MI rats, which were indicative of improvement in conduction (Fig. 4C). Representative tracings for TAT dispersion in the four groups are presented in Fig. 4B. Compared with the sham-operated hearts, the hearts with MI exhibited prolonged TAT dispersion (Fig. 4B and C). Exendin-4 treatment restored TAT dispersion to a level similar to that of the control value (Fig. 4B and C), and there was no significant difference between sham rat and MI+Ex-4 rats in TAT dispersion. As shown in Fig. 4A, the activation pattern was uniform in the sham-operated hearts; however, the activation pattern was more heterogeneous in the hearts with MI. Exendin-4 treatment ameliorated the anisotropic conduction properties. However, the GLP-1R antagonist partly diminished the beneficial effects of exendin-4 on excitation transmission.

Exendin-4 suppresses atrial fibrosis. As atrial fibrosis is one of the main atrial structural alterations responsible for the maintenance and progression of AF (19), the expression levels of profibrotic markers in the atrium were analyzed. Specifically, Col I, Col III and TGF-β1 expression levels were examined using western blotting and the CVF was assessed by Masson's trichrome staining. It was demonstrated that the Col I, Col III and TGF-β1 protein expression levels, and the CVF, were higher in the rats with MI compared with those who underwent the sham operation (Fig. 5A and B). However, these increases were attenuated by exendin-4 treatment (Fig. 5A and B). The expression of Col I, Col III and TGF-β1 protein, and the CVF were increased in MI+Ex-4+Ex9-39 rats compared with MI+Ex-4 rats. Thus, the inhibition of GLP-1R by exendin9-39 partly diminished the suppressive effects of exendin-4 on atrial fibrosis (Fig. 5A and B).

Exendin-4 inhibits the PI3K/AKT signaling pathway activity. To determine whether the GLP-1 receptor plays a role in atrial arrhythmogenesis in the MI-induced heart failure model, atrial GLP-1 receptor expression and plasma GLP-1 levels were assessed using western blotting analysis and ELISA. The plasma GLP-1 levels and atrial *GLP-1* receptor mRNA and protein expression levels were significantly downregulated following MI (Fig. 6A and B). Exendin-4 treatment increased the plasma GLP-1 levels, and the atrial *GLP-1* receptor mRNA and protein expression levels (Fig. 6A-C).

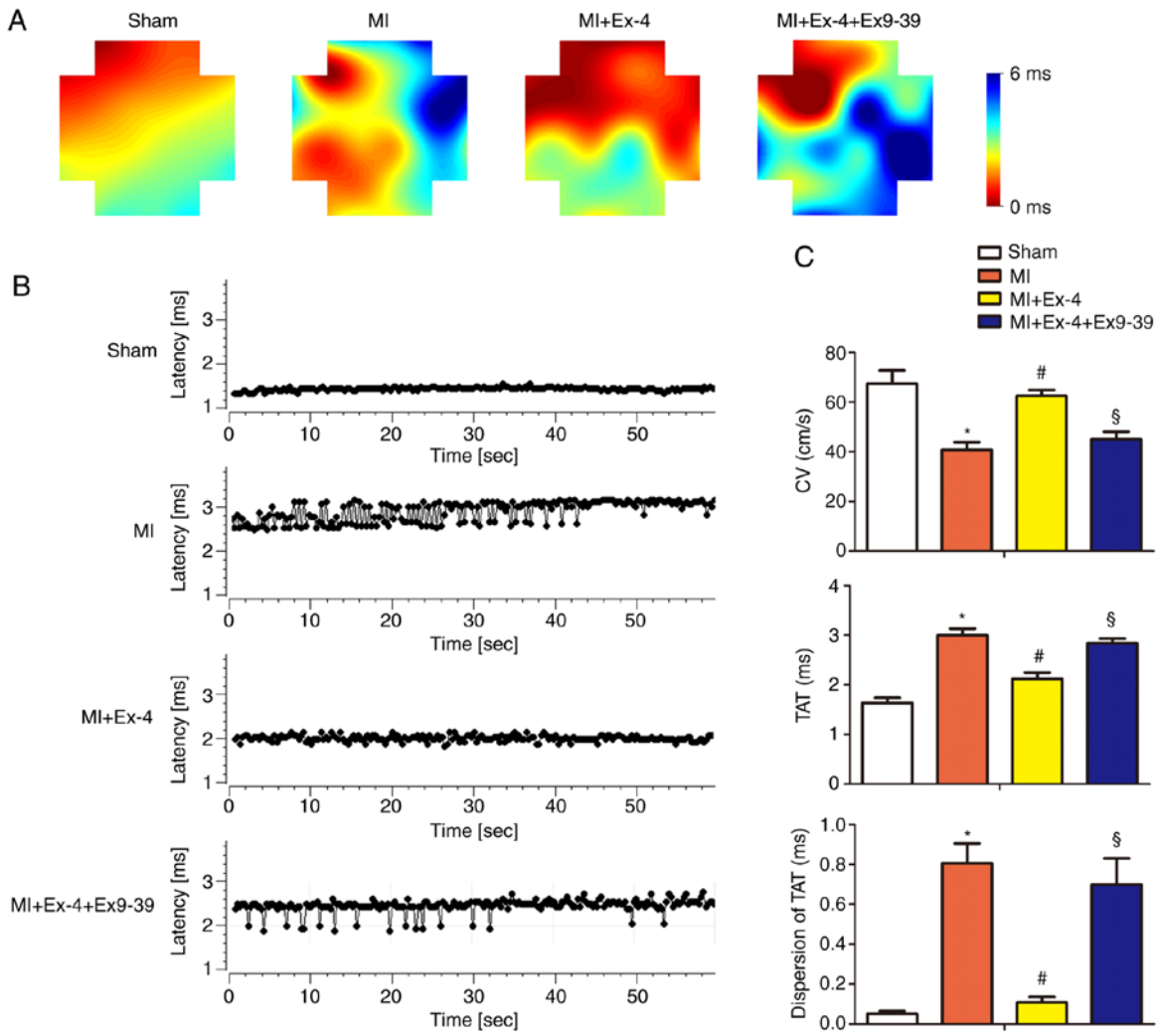

Figure 4. Ex-4 attenuates conduction slowing and conduction heterogeneity in the left atrial appendage following myocardial infarction. (A) Representative recordings of maps in each group. (B) Representative long-term traces of instantaneous-beat TATs. (C) Statistical analysis of conduction velocity, total activation time and TAT dispersion in the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 rat hearts 4 weeks following sham or MI surgery (n=5 animals per group). *P<0.05 vs. the sham-operated group, #P<0.05 vs. the MI group, §P<0.05 versus the MI+Ex-4 group. MI, myocardial infarction; Ex-4, exendin-4; Ex9-39, exendin9-39; TAT, total activation time; CV, conduction velocity.

However, these effects were partly neutralized by co-treatment with exendin9-39 (Fig. 6A-C).

To investigate the mechanisms underlying the effects of exendin-4 on atrial fibrosis, the PI3K/AKT signaling pathway was assessed. Compared with the atrial tissues from sham-operated rats, the tissues from rats with MI exhibited increased phosphorylated PI3K and AKT levels compared with sham rats, although the total PI3K and AKT levels remained unaltered between the two groups. The increased expression levels of phosphorylated PI3K and AKT were attenuated by exendin-4 treatment (Fig. 6B and C), and all the effects of exendin-4 were neutralized by the co-administration of exendin9-39 (Fig. 6B and C).

Discussion

To the best of our knowledge, the present study is the first to demonstrate the effects of exendin-4 on atrial fibrosis and atrial arrhythmogenesis susceptibility. The results demonstrated that in rats with MI-induced heart failure, atrial fibrosis and atrial arrhythmogenesis susceptibility were increased. In addition, increased atrial tachyarrhythmias susceptibility, reductions in conduction velocity and increases in conduction dispersion were observed. Treatment with exendin-4 attenuated atrial fibrosis and atrial arrhythmogenesis susceptibility. The co-administration of exendin9-39 and exendin-4 partly diminished the protective effects of exendin-4. The increased atrial arrhythmogenesis susceptibility was associated with atrial fibrosis, which partially induced by the enhanced phosphorylation of the PI3K/AKT signaling pathway, an effect that was abolished by exendin-4. The beneficial effects of exendin-4 were attenuated by the co-administration of the GLP-1 receptor antagonist, exendin9-39, and exendin-4. Taken together, these findings indicated that exendin-4 attenuates atrial fibrosis at least partly through PI3K/AKT signaling pathway inactivation via the GLP-1 receptor and inhibits atrial arrhythmogenesis.

The cardioprotective effects of exendin-4 are well known and a previous study by the authors demonstrated the cardioprotective effects of exendin-4 on a rat model of chronic MI through the endothelial nitric oxide synthase/cyclic guanosine monophosphate/protein kinase G pathway (9). Our previous

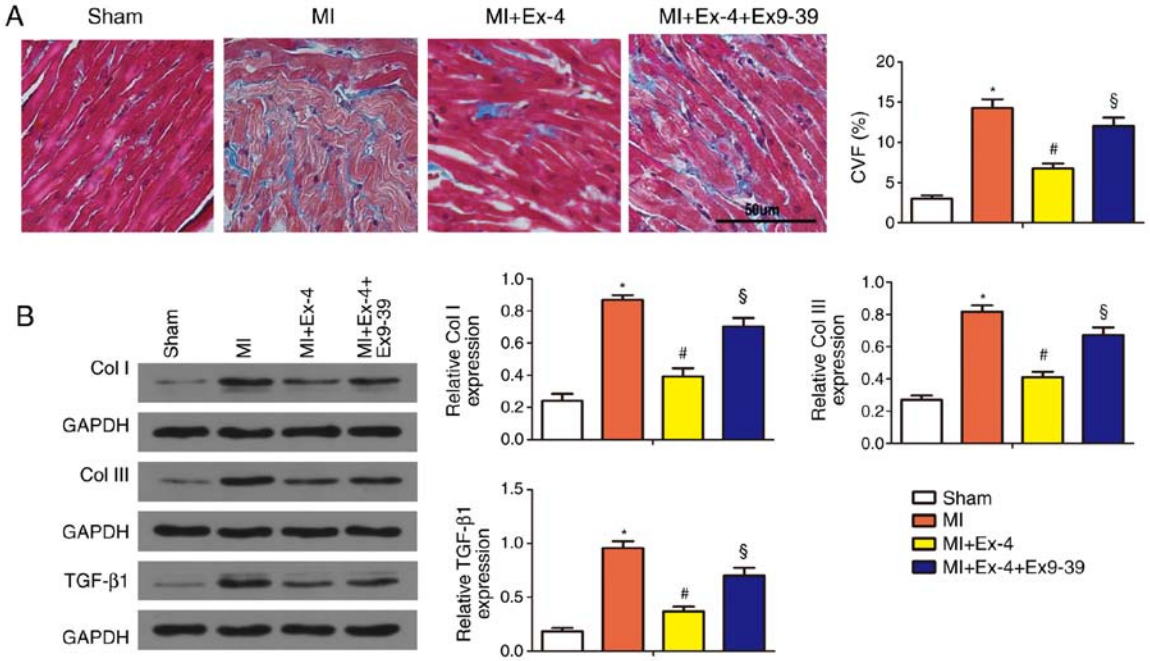

Figure 5. Ex-4 attenuates atrial fibrosis and fibrotic markers expression in the left atrial appendage. (A) Interstitial fibrosis was evaluated using Masson's staining for collagen and quantitative analysis of the CVF. (B) Representative western blots of atrial tissue lysates probed for Col I, Col III and TGF-β1 expression in the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 rat hearts 4 weeks following sham or MI surgery and statistical analysis of the protein expression levels of these proteins, which were normalized to those of GAPDH (n=5 animals per group). *P<0.05 vs. the sham-operated group, #P<0.05 vs. the MI group, §P<0.05 vs. the MI+Ex-4 group. MI, myocardial infarction; Ex-4, exendin-4; Ex9-39, exendin9-39; CVF, collagen volume fraction; Col, collagen; TGF, transforming growth factor.

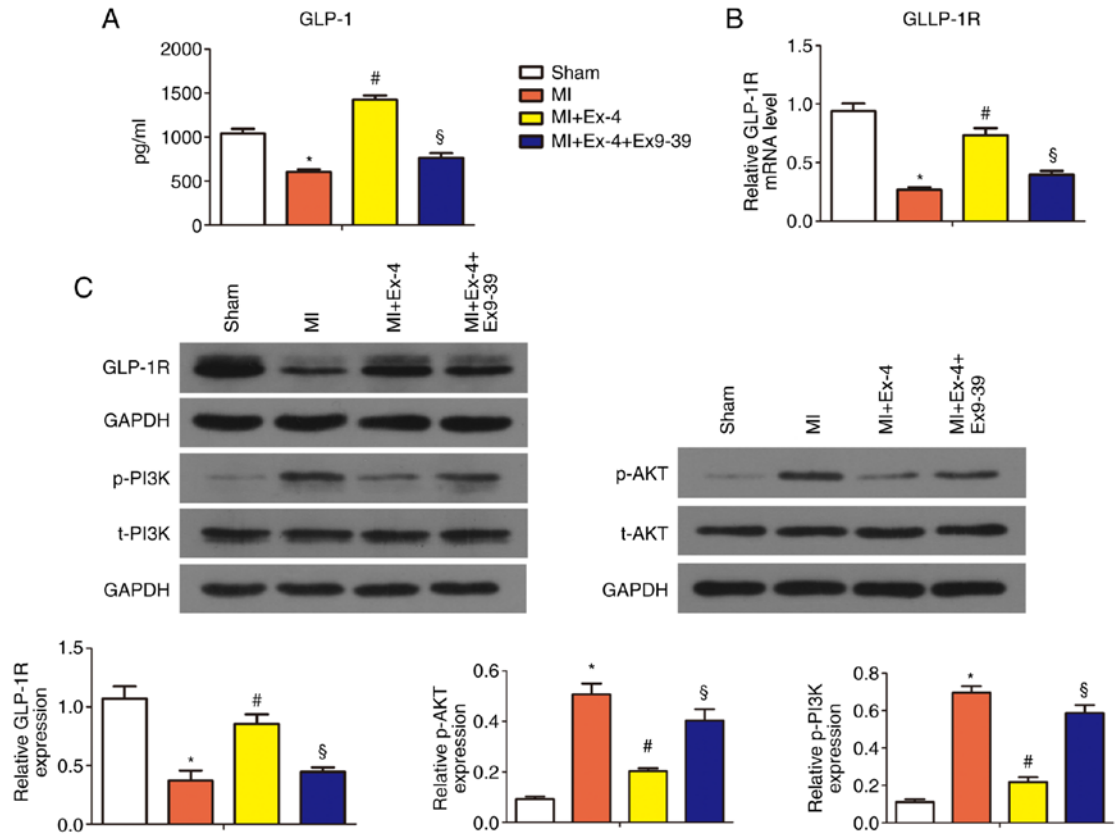

Figure 6. Exendin-4 inhibits the PI3K/AKT signaling pathway. (A) Plasma GLP-1 levels and (B) *GLP-1* receptor mRNA expression as determined by reverse transcription-quantitative PCR in the sham-operated, MI, MI+Ex-4 and MI+Ex-4+Ex9-39 rat atrial tissues 4 weeks following sham or MI surgery. (C) Representative western blots of atrial tissues probed for GLP-1 receptor, total PI3K, phosphorylated PI3K, total AKT and phosphorylated AKT in the sham-operated, MI, MI+Exendin-4 and MI+Exendin-4+Ex9-39 rat hearts four weeks following sham or MI surgery and statistical analysis of the expression levels of these proteins, which were normalized to those of GAPDH (n=5 animals per group). *P<0.05 vs. the sham-operated group, #P<0.05 vs. the MI group, §P<0.05 vs. the MI+Ex-4 group. MI, myocardial infarction; Ex-4, exendin-4; Ex9-39, exendin9-39; GLP-1, glucagon-like peptide-1 receptor; p-, phosphorylated; t-, total.

studies showed that exendin-4 has direct electrophysiological effect on electrocardiogram parameters including QRS, QT and QTc intervals and calcium handling in ventricular arrhythmias (9,20). However, limited information is available regarding the effects of exendin-4 on atrial electrophysiology in heart failure. Previous studies have primarily focused on the effects of exendin-4 on ventricular structural remodeling (6,9). In the present study, the effects of exendin-4 on atrial arrhythmogenesis were investigated in a model of MI-induced heart failure. Sitagliptin (a DPP-4 inhibitor) has been shown to attenuate ventricular arrhythmias in rats with MI by inhibiting sympathetic innervation (21). Chinda *et al* (22) reported that the administration of vildagliptin (another DPP-4 inhibitor) prior to LAD occlusion in a rat ischemia/reperfusion model, modulated cardiac electrophysiology by normalizing the shortening of the effective refractory period, decreasing the occurrence of premature ventricular contractions and increasing the ventricular fibrillation threshold during the ischemic period. A previous study demonstrated that GLP-1 (10 nM) increased calcium transients and sarcoplasmic reticular Ca^{2+} contents by regulating the expression of calcium handling proteins. Specifically, GLP-1 decreased total phospholamban expression and ryanodine receptor phosphorylation at S2814 in HL-1 cardiomyocytes (23). GLP-1 has been shown to exert direct effects on atrial myocyte electrophysiology (23); however, no studies to date have focused on the effects of exendin-4 on atrial electrophysiological properties in a heart failure model, at least to the best of our knowledge. Li *et al* (24) demonstrated that atrial APDs were prolonged in heart failure, a finding consistent with that observed herein. In the present study, it was shown that exendin-4 treatment shortened APDs. Moreover, it was reported that exendin-4 treatment shortened APDs, and decreased atrial tachyarrhythmias inducibility in the rat model of MI-induced heart failure. As chronic heart failure creates a substrate for AF (24,25), the beneficial effects of exendin-4 on heart failure-induced atrial arrhythmias may be partly responsible for the improvements in cardiac function elicited by the drug. Furthermore, the effects of exendin-4 on atrial arrhythmogenesis were independent of glucose control in a previous study (4).

In dogs with heart failure, interstitial fibrosis, slow conduction and increased conduction heterogeneity result in an increased susceptibility to AF. The key atrial electrophysiology alterations that occur in chronic heart failure may be the caused by interstitial fibrosis-induced changes in atrial conduction (25). Local atrial conduction abnormalities interrupt cardiac electrical continuity, resulting in unidirectional conduction slowing, which may promote reentry maintenance (26,27). In the present study, slow conduction and increased TAT dispersion were observed in the rat model of MI-induced heart failure. Exendin-4 treatment increased conduction velocity and decreased TAT dispersion, and these changes may underlie the stabilizing effects of this treatment on atrial electrophysiology.

Atrial fibrosis is a major cause of AF and a strong predictor of the clinical outcomes of patients with heart failure. Atrial fibrosis-induced conduction alterations have been shown to be a major substrate for patients with AF (19). Additionally, atrial fibrosis favors unidirectional conduction slowing or blockage, which leads to reentry initiation and re-entry maintenance (28). Given that the results of several studies have indicated that atrial fibrosis facilitates AF (27,28), it is reasonable to hypothesize that exendin-4 reduces atrial fibrillation susceptibility by attenuating atrial fibrosis.

A previous study demonstrated that TGF-β1 functions as a fibrogenic cytokine by stimulating collagen synthesis, resulting in increased Col I and Col III expression (29). A previous study demonstrated that chronic treatment with angiotensin II increased TGF-β expression levels, a change associated with worse cardiac fibrosis and enhanced cardiac collagen synthesis (30). In the present study, TGF-β1 expression was increased in the MI-induced heart failure model, and exendin-4 attenuated TGF-β1 expression, a change accompanied by decreases in Col I and Col III expression, suggesting that atrial fibrosis is modulated by exendin-4. These results were consistent with those of a previous study (31), in which the treatment of macrophages with exendin-4 attenuated the increased expression of several cytokines (α-smooth muscle actin, connective tissue growth factor and TGF-β1) under high glucose conditions and TGF-β stimulation.

The GLP-1 receptor has been reported to be located in atrial myocytes and arteries; however, it is not expressed in fibroblasts (8), which suggests that the effects of exendin-4 on atrial fibrosis may occur secondary to paracrine signaling. A previous study demonstrated that exendin-4 had no effect on the TGF-β-induced differentiation of cardiac fibroblasts, indicating that the effects of exendin-4 on atrial fibrosis may be mediated by another cell type (32). Tate *et al* (31) reported that exendin-4 exerted an effect on paracrine communication between macrophages and cardiac fibroblasts, which may provide an explanation for the observation of the present study. It was also demonstrated that GLP-1 receptor expression levels were decreased in atrial tissues following MI and were normalized by exendin-4 treatment (31).

A previous study focusing on the acute cardioprotective effects of exendin-4 in a rat ischemia/reperfusion model demonstrated that PI3K/AKT signaling pathway activation exerted cardioprotective effects attributable to its pro-survival and antiapoptotic effects (12). However, chronic cardiomyocyte-specific AKT activation has been shown to promote both pathological hypertrophy and fibrosis. Transgenic mice with cardiac-specific AKT-overexpression exhibit cardiac hypertrophy at the molecular and histological levels (33). Moreover, $Akt1^{-/-}$ mice have been shown to be protected from hypoxia and TGF-β-induced lung fibrosis, while Akt-overexpressing mice exhibit pulmonary vascular fibrosis (34). The findings of aforementioned studies support the hypothesis that exendin-4-induced PI3K/AKT signaling pathway inactivation may contribute to the effects of exendin-4 on atrial fibrosis. In the present study, the role of the PI3K/AKT signaling pathway in the development of atrial fibrosis was investigated and it was found that rats with MI exhibited increased PI3K/AKT phosphorylation in atrial tissue, and abnormalities that were attenuated by treatment with exendin-4; however, these effects were partly abolished by co-administration with exendin9-39. These data suggested that the PI3K/AKT signaling pathway is the downstream target of the GLP-1 receptor in atrial tissues in the MI-induced heart failure model.

There are several limitations to the present study. Firstly, only the electrophysiology in LA responding to exendin-4

was assessed. As LA was the main substrate of AF, only the response of LA to exendin-4 treatment was measured. Future studies should explore the difference between RA and LA responding to extenin-4. Atrial conduction and atrial fibrosis were aspects of atrial arrhythmogenesis, as well as ionic current and calcium handling. In future studies, the effects of exendin-4 on ionic current and calcium handling should be explored.

In conclusion, the results of the present study demonstrated that the GLP-1 receptor agonist, exendin-4, decreased atrial fibrosis and susceptibility to atrial arrhythmogenesis in the rat model of MI-induced heart failure. The beneficial effects of exendin-4 were partially diminished by the co-administration of exendin9-39. Moreover, it was shown that exendin-4 exerted its antifibrotic effects partly by inhibiting the PI3K/AKT signaling pathway via the GLP-1 receptor. These data provide new insight regarding the association between atrial electrophysiology and exendin-4.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81960047), Science and Technology Foundation of Guiyang City [grant no. (2019)-9-1], the Science and Technology Foundation of Health commission of Guizhou Province (grant no. gzwjkj2019-1-092), and the Science and Technology Foundation of Guizhou Provence [grant no. (2019)2800].

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

WL conceived, designed and supervised the present study. JinC and SX performed the experiments, reviewed the manuscript, and analyzed and interpreted the data. LoW, WZ, PL, ND, QT, YL, LiW and JiuC performed the experiments. All authors read and approved the fnal manuscript.

Ethics approval and consent to participate

All experimental protocols were approved by the Experimental Animal Care and Institutional Animal Ethical Committee of Guizhou Medical University (Guiyang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kemp CD and Conte JV: The pathophysiology of heart failure. Cardiovasc Pathol 21: 365-371, 2012.
2. Lau DH, Schotten U, Mahajan R, Antic NA, Hatem SN, Pathak RK, Hendriks JM, Kalman JM and Sanders P: Novel mechanisms in the pathogenesis of atrial fibrillation: Practical applications. Eur Heart J 37: 1573-1581, 2016.
3. Verheule S, Eckstein J, Linz D, Maesen B, Bidar E, Gharaviri A and Schotten U: Role of endo-epicardial dissociation of electrical activity and transmural conduction in the development of persistent atrial fibrillation. Prog Biophys Mol Biol 115: 173-185, 2014.
4. Robinson E, Cassidy RS, Tate M, Zhao Y, Lockhart S, Calderwood D, Church R, McGahon MK, Brazil DP, McDermott BJ, *et al*: Exendin-4 protects against post-myocardial infarction remodelling via specific actions on inflammation and the extracellular matrix. Basic Res Cardiol 110: 20, 2015.
5. Noyan-Ashraf MH, Momen MA, Ban K, Sadi AM, Zhou YQ, Riazi AM, Baggio LL, Henkelman RM, Husain M and Drucker DJ: GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. Diabetes 58: 975-983, 2009.
6. DeNicola M, Du J, Wang Z, Yano N, Zhang L, Wang Y, Qin G, Zhuang S and Zhao TC: Stimulation of glucagon-like peptide-1 receptor through exendin-4 preserves myocardial performance and prevents cardiac remodeling in infarcted myocardium. Am J Physiol Endocrinol Metab 307: E630-E643, 2014.
7. Bao W, Aravindhan K, Alsaid H, Chendrimada T, Szapacs M, Citerone DR, Harpel MR, Willette RN, Lepore JJ and Jucker BM: Albiglutide, a long lasting glucagon-like peptide-1 analog, protects the rat heart against ischemia/reperfusion injury: Evidence for improving cardiac metabolic efficiency. PLoS One 6: e23570, 2011.
8. Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S, Gribble FM and Reimann F: Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. Diabetes 63: 1224-1233, 2014.
9. Chen J, Wang D, Wang F, Shi S, Chen Y, Yang B, Tang Y and Huang C: Exendin-4 inhibits structural remodeling and improves Ca2+ homeostasis in rats with heart failure via the GLP-1 receptor through the eNOS/cGMP/PKG pathway. Peptides 90: 69-77, 2017.
10. Iwasa M, Kobayashi H, Yasuda S, Kawamura I, Sumi S, Yamada Y, Shiraki T, Yamaki T, Ushikoshi H, Aoyama T, *et al*: Antidiabetic drug voglibose is protective against ischemia-reperfusion injury through glucagon-like peptide 1 receptors and the phosphoinositide 3-kinase-Akt-endothelial nitric oxide synthase pathway in rabbits. J Cardiovasc Pharmacol 55: 625-634, 2010.
11. Wende AR, O'Neill BT, Bugger H, Riehle C, Tuinei J, Buchanan J, Tsushima K, Wang L, Caro P, Guo A, *et al*: Enhanced cardiac Akt/protein kinase B signaling contributes to pathological cardiac hypertrophy in part by impairing mitochondrial function via transcriptional repression of mitochondrion-targeted nuclear genes. Mol Cell Biol 35: 831-846, 2015.
12. Basalay MV, Mastitskaya S, Mrochek A, Ackland GL, Del AA, Sanchez J, Sjoquist PO, Pernow J, Gourine AV and Gourine A: Glucagon-like peptide-1 (GLP-1) mediates cardioprotection by remote ischaemic conditioning. Cardiovasc Res 112: 669-676, 2016.
13. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US), 2011.
14. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ and Elmquist JK: Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J Clin Invest 110: 43-52, 2002.
15. Sisley S, Smith K, Sandoval DA and Seeley RJ: Differences in acute anorectic effects of long-acting GLP-1 receptor agonists in rats. Peptides 58: 1-6, 2014.
16. Wang D, Liu T, Shi S, Li R, Shan Y, Huang Y, Hu D and Huang C: Chronic administration of catestatin improves autonomic function and exerts cardioprotective effects in myocardial infarction rats. J Cardiovasc Pharmacol Ther 21: 526-535, 2016.
17. Amino M, Yoshioka K, Tanabe T, Tanaka E, Mori H, Furusawa Y, Zareba W, Yamazaki M, Nakagawa H, Honjo H, *et al*: Heavy ion radiation up-regulates Cx43 and ameliorates arrhythmogenic substrates in hearts after myocardial infarction. Cardiovasc Res 72: 412-421, 2006.

18. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
19. Nattel S: Molecular and cellular mechanisms of atrial fibrosis in atrial Fibrillation. JACC Clin Electrophysiol 3: 425-435, 2017.
20. Chen J, Xu S, Zhou W, Wu L, Wang L and Li W: Exendin-4 reduces ventricular arrhythmia activity and calcium sparks-mediated sarcoplasmic reticulum Ca Leak in rats with heart failure. Int Heart J 61: 145-152, 2020.
21. Lee TM, Chen WT and Chang NC: Sitagliptin decreases ventricular arrhythmias by attenuated glucose-dependent insulinotropic polypeptide (GIP)-dependent resistin signalling in infarcted rats. Biosci Rep 36: e00307, 2016.
22. Chinda K, Palee S, Surinkaew S, Phornphutkul M, Chattipakorn S and Chattipakorn N: Cardioprotective effect of dipeptidyl peptidase-4 inhibitor during ischemia-reperfusion injury. Int J Cardiol 167: 451-457, 2013.
23. Huang JH, Chen YC, Lee TI, Kao YH, Chazo TF, Chen SA and Chen YJ: Glucagon-like peptide-1 regulates calcium homeostasis and electrophysiological activities of HL-1 cardiomyocytes. Peptides 78: 91-98, 2016.
24. Li D, Melnyk P, Feng J, Wang Z, Petrecca K, Shrier A and Nattel S: Effects of experimental heart failure on atrial cellular and ionic electrophysiology. Circulation 101: 2631-2638.22, 2000.
25. Li D, Fareh S, Leung TK and Nattel S: Promotion of atrial fibrillation by heart failure in dogs: Atrial remodeling of a different sort. Circulation 100: 87-95, 1999.
26. Lammers WJ, Schalij MJ, Kirchhof CJ and Allessie MA: Quantification of spatial inhomogeneity in conduction and initiation of reentrant atrial arrhythmias. Am J Physiol 259: H1254-H1263, 1990.
27. Jalife J and Kaur K: Atrial remodeling, fibrosis, and atrial fibrillation. Trends Cardiovasc Med 25: 475-484, 2015.
28. Vigmond E, Pashaei A, Amraoui S, Cochet H and Hassaguerre M: Percolation as a mechanism to explain atrial fractionated electrograms and reentry in a fibrosis model based on imaging data. Heart Rhythm 13: 1536-1543, 2016.
29. Purnomo Y, Piccart Y, Coenen T, Prihadi JS and Lijnen PJ: Oxidative stress and transforming growth factor-β1-induced cardiac fibrosis. Cardiovasc Hematol Disord Drug Targets 13: 165-172, 2013.
30. Zhao W, Zhao T, Chen Y, Ahokas RA and Sun Y: Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. Mol Cell Biochem 317: 43-50, 2008.
31. Tate M, Robinson E, Green BD, McDermott BJ and Grieve DJ: Exendin-4 attenuates adverse cardiac remodelling in streptozocin-induced diabetes via specific actions on infiltrating macrophages. Basic Res Cardiol 111: 1, 2016.
32. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ and Husain M: Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 117: 2340-2350, 2008.
33. Condorelli G, Drusco A, Stassi G, Bellacosa A, Roncarati R, Iaccarino G, Russo MA, Gu Y, Dalton N, Chung C, *et al*: Akt induces enhanced myocardial contractility and cell size in vivo in transgenic mice. Proc Natl Acad Sci USA 99: 12333-12338, 2002.
34. Abdalla M, Sabbineni H, Prakash R, Ergul A, Fagan SC and Somanath PR: The Akt inhibitor, triciribine, ameliorates chronic hypoxia-induced vascular pruning and TGFβ-induced pulmonary fibrosis. Br J Pharmacol 172: 4173-4188, 2015.