Department of Cardiology[1], First Affiliated Hospital of Xi'an Jiaotong University, Xi'an; Department of Cardiology[2], Jinan Central Hospital Affiliated to Shandong University, Jinan, China

Ginsenoside Rg1 inhibits myocardial ischaemia and reperfusion injury *via* HIF-1α-ERK signalling pathways in a diabetic rat model

Chengjun Yuan[1], Huating Wang[2], Zuyi Yuan[1,*]

Received November 7, 2018, accepted December 12, 2018

**Corresponding author: Prof. Dr. Zuyi Yuan, Department of Cardiology, First Affiliated Hospital of Xi'an Jiaotong University, Yan Ta West Road 277, Xi'an, 710061, Shaanxi, China.*
zuyiyuanca@gmail.com

Pharmazie 74: 157-162 (2019) *doi: 10.1691/ph.2019.8858*

The aim of this study was to observe the effects of HIF-1α activation on myocardial I/R in diabetes. Diabetes was induced in an experimental rat model, and regulators of HIF-1α including KC7F2, deferoxamine and ginsenoside Rg1 were administered to observe the changes on diabetic rats. The results demonstrated that HIF-1α activation could effectively reduce myocardial injury following I/R in diabetic hearts *via* ERK but not MMP-2 signalling pathways. This activation promoted myocardial apoptosis, which was accompanied by modulation of Bax/Bcl-2, caspase-3 and caspase-9 expression following deferoxamine administration. Ginsenoside Rg1 application but not Re can activate HIF-1α, resulting in a similar protectively effect on these pathology processes. Our data demonstrated that ginsenoside Rg1 has a potential therapeutic effect by protecting diabetic hearts after myocardial injury following I/R via HIF-1α activation.

1. Introduction

Diabetes patients are at major risk for cardiovascular morbidity and mortality, which can be caused by ischaemia and reperfusion (I/R) injury according to pathological and epidemiological evidence (Miki et al. 2013; Messaoudi et al. 2013). Based on previous clinical studies (Yin et al. 2012; Kataoka et al. 2012), the mortality of diabetic patients 5 years after myocardial infarction is approximately 50 %, which is much higher than that of non-diabetic patients. It was reported that the exaggerated size of infarcts observed in diabetes patients could explain their poor prognoses and could be the main cause of diabetes-related death following I/R (Brown 2012; Kravchuk et al. 2011; Miki et al. 2012). Therefore, it is important to investigate the molecular mechanisms underlying this pathological process and to develop new strategies for combating diabetes-induced aggravation of ischemia and reperfusion (I/R) injury.

It has been demonstrated that the diabetic heart is susceptible to I/R injury due to increased oxidative stress. Additionally, diabetes-related gene expression and regulation (e.g., inactivation or activation) can induce certain epigenetic and genetic changes that influence the progression or development of I/R injury (Das et al. 2014; Tao et al. 2007). Previous studies have suggested that myocardial apoptosis was the main pathological indicator of myocardial injury, thus inhibition on myocardial apoptosis would limit myocardial injury and the development of cardiac remodeling and dysfunction (DeBerge et al. 2017; Lejay et al. 2016).

Hypoxia inducible factor (HIF), which includes the HIF-1α and HIF-1β subunits, is primarily regulated by oxygen. Additionally, important oxygen-modulated genes that are critical in both diabetes pathology and I/R injury can be regulated via the HIF-1α signalling pathway (Bohuslavova et al. 2014, 2013). Therefore, HIF-1α is viewed as a potential target for reducing the aggravation of I/R injury induced by diabetes. However, there are no available reports describing the effects of HIF-1α on the regulation of myocardial ischaemia in diabetic rats, and the underlying mechanism and related signalling pathways remain unclear. In addition, using a specific modulator can effectively regulate the expression of target genes — a relationship that has been demonstrated in various clinical diseases (Nishimoto et al. 2014; Sen et al. 2013). Therefore, modulation at the level of HIF-1α may help to identify novel potential therapeutic targets for the treatment of I/R injury in diabetic individuals, while allowing us to elucidate the mechanisms underlying disease development.

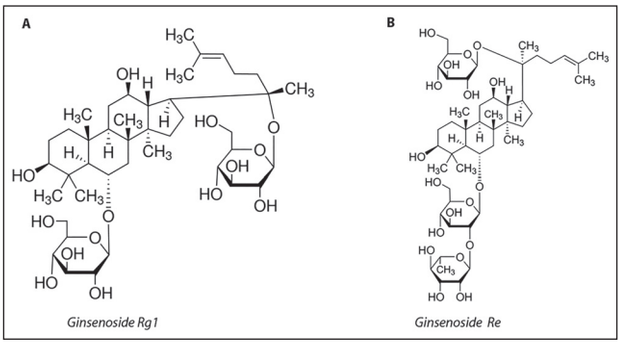

Fig. 1: Chemical structures of ginsenoside Rg1 (A), Ginsenoside Re (B).

Ginsenosides Rg1 and Re (Fig. 1) have the different effects on cell biological behavior. Rg1 increases the density of newly formed vessels, and enhances VEGF expression levels but reduces p38 MAPK by activating PI3K/Akt due to its steroid hormone-like structure. Re reduces the inflammatory effects and decreases inflammatory cytokine expression by inhibiting LPS action (Kim et al. 2017; Lu et al. 2009; Radad et al. 2004). In addition, Ginsenoside Rg1 can affect the myocardial ischaemia and reperfusion injury but the underlying mechanism is still not clear (Miswan et al. 2016; Deng et al. 2015; Zhang et al. 2012). Thus, the purpose of this study was to test the hypothesis that the positive or negative modulation of ginsenoside on HIF-1α plays a crucial role in the regulation of I/R injury in the diabetic heart. Furthermore, we attempted to elucidate the mechanism by which increased bioavailability of HIF-1α might protect the diabetic heart.

Table: Effects on basic parameters

Parameters	Control	I/R	Diabetic+ I/R	KC7F2	Re	Rg1	Deferoxamine
Arterial pressure (mmHg)	101.5 ± 3.1	110.4 ± 3.3	107.8 ± 2.6	110.2 ± 4.2	106.8 ± 1.9	107.2 ± 3.1	108.6 ± 2.5
Blood glucose (mM)	29.5 ± 1.2	32.3 ± 2.1	56.5 ± 1.5	63.2 ± 2.1	57.1 ± 1.2	40.3 ± 3.1**	36.2 ± 1.5**
Heart rate, beats/min	368 ± 5	382 ± 6	390 ± 3	386 ± 3	381 ± 3	379 ± 5	380 ± 3
Body weight (g)	268.2 ± 1.2	270.1 ± 2.3	275.2 ± 1.3	268.5 ± 1.4	273.5 ± 3.3	271.3 ± 3.0	268.4 ± 2.5
Heart weight (g)	8.3 ± 0.6	8.4 ± 0.4	9.0 ± 0.2	9.2 ± 0.4	8.9 ± 0.1	9.1 ± 0.5	8.7 ± 0.2
Heart /body ratio (%)	3.0 ± 0.1	3.2 ± 0.4	3.1 ± 0.3	3.0 ± 0.3	2.9 ± 0.4	2.8 ± 0.6	3.0 ± 0.3

*(**P < 0.01, n = 6, vs Diabetic + I/R group)*

2. Investigations and results

2.1. Effect on myocardial infarct size

The basic parameters assessed in this work after above treatment are shown in the Table. The blood glucose was reduced after administration of deferoxamine or ginsenoside Rg1 compared with that in the I/R group (P<0.01, n=6). The reduction in myocardial infarct size was evaluated after administration with different chemicals to observe the changes on myocardial injury following I/R in diabetic hearts. As shown in Fig. 2, the infarct size (% risk area) in the diabetic hearts was significantly increased compared with that in the I/R group (60.0±1.7 % vs. 72.6±2.8 %, P<0.01; n=6). After administration of ginsenoside Rg1, the infarct size was markedly reduced (51.8±2.0 %, P < 0.01; n = 6) but not in the group of ginsenoside Re group (70.0±1.9 %, P >0.05; n = 6). In addition, the infarct size was significantly smaller in the group receiving a dose of 50 μM deferoxamine (41.6±2.9 %, P < 0.01; n = 6), which can specifically activate HIF-1α, but no difference was observed in the group with 20 μM KC7F2 administration. The above data showed that HIF-1α activation can efficiently decrease the size of myocardial injury in diabetic hearts, and a similar effect was obtained in the deferoxamine group compared with the ginsenoside Rg1 group. Based on these results, it was interesting that only ginsenoside Rg1 but not Re could be the potent activator of HIF-1α. The subsequent experiments were conducted to obtain further evidence of the possible mechanism underlying this effect.

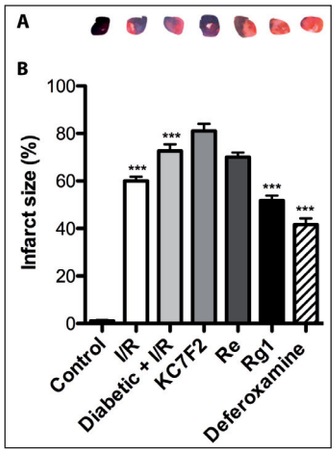

Fig. 2: Effect on myocardial infarct size. The reduction of myocardial infarct size was evaluated after the following administration: control (control), ischaemia and reperfusion injury (I/R), ischaemia and reperfusion injury in diabetic rats (diabetic + I/R), ischaemia and reperfusion injury in diabetic rats after the administration of a HIF-1α inhibitor (KC7F2), ginsenoside Rg1 (Rg1), ginsenoside Re (Re) and HIF-1α activator (deferoxamine). Bars indicate the standard deviation of the mean.

2.2. Effect on plasma enzyme activities in diabetic rats

Plasma CK, LDH, AST and ALT activities were measured to evaluate the effect of ginsenoside on myocardial injury following I/R in diabetic hearts. As shown in Fig. 3, the CK level in diabetic hearts was significantly increased compared with that in the hearts of the I/R group (2,580±96 U/L vs. 1,858±133 U/L, P<0.01; n=6), and after the administration of deferoxamine and ginsenoside Rg1, the CK levels were reduced (1,538±131 U/L, P<0.001, n=6; 1,678±52 U/L, P<0.01, n=6). Furthermore, LDH activity in the diabetic hearts was significantly increased compared with that in the hearts of the I/R group (2,000±60 U/L vs. 1,507±105 U/L, P<0.01; n=6), and after the administration of deferoxamine or ginsenoside Rg1, LDH activity was significantly reduced (1,438±64 U/L, P<0.001,n=6; 1,658±144 U/L, P<0.001, n=6). The activities of AST and ALT were also significantly reduced (999±47 U/L vs. 1,683±88 U/L and 950±54 U/L vs. 1,880±125 U/L, respectively; P<0.001; n=6) after the administration of deferoxamine, and a similar tendency was obtained for the ginsenoside Rg1 group (1259±95 U/L vs. 1,683±88 U/L and 1051±88 U/L vs. 1,880±125 U/L, respectively; P<0.001; n=6). Interestingly the above results clearly showed downregulation of plasma enzyme activities in diabetic rats was not observed in the ginsenoside Re group. These results demonstrated that HIF-1α activation by the direct activator could reduce myocardial injury following I/R in diabetic hearts via positively or negatively modulating the activities of CK, LDH, AST and ALT in plasma. Ginsenoside Rg1 but not Re could also induce the above similar effect via a possible influence on HIF-1α activation.

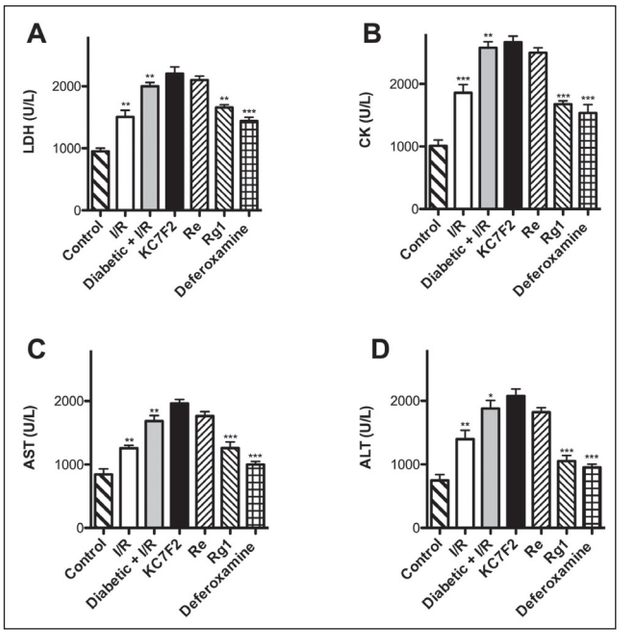

Fig. 3: Effect on plasma enzyme activities in diabetic rats. Plasma (A) LDH, (B) CK, (C) AST and (D) ALT activities were measured to evaluate the effect of HIF-1α activation on myocardial injury following I/R in diabetic hearts using a specific analysis kit. Bars indicate the standard deviation of the mean.

2.3. Effect on myocardium apoptosis in diabetic rats

To test the effect of HIF-1α activation on myocardial apoptosis in diabetic hearts, myocardial apoptosis was observed through TUNEL assays in the following experiments. Fig. 4A shows that the percentage of TUNEL-positive myocyte nuclei in diabetic hearts was significantly increased compared with that in the hearts of the I/R group, and after treatment with deferoxamine, the percentage of TUNEL-positive myocyte nuclei in diabetic hearts

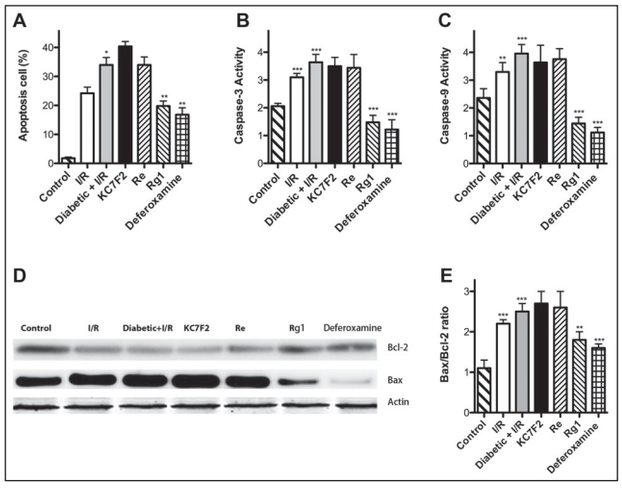

Fig. 4: Effect on myocardial apoptosis in diabetic rats. (A) Myocardial apoptosis was tested *via* TUNEL assays with an apoptosis detection kit; (B) Caspase-3 and (C) caspase-9 activities were also measured with an assay kit; (D) and (E) BAX / Bcl-2 levels were analyzed by Western blotting in diabetic rats after treatment with KC7F2 or deferoxamine. β-actin levels are shown as a loading control.

was significantly reduced (16.8±2.3 % vs. 34.0±2.5 %, P<0.01; n=6). Downregulation after the above treatment (19.8±1.7 % vs. 34.0±2.5 %, P<0.01; n=6) was observed in the Rg1 group, but no changes were observed in the ginsenoside Re group.

In addition, caspase-3 and caspase-9 activities were measured with an assay kit. As shown in Fig. 4B, 4C, caspase-3 and caspase-9 activities in diabetic hearts were significantly increased compared with that in the hearts of the control group, whereas after the administration of deferoxamine, their activities were markedly reduced. Ginsenoside Rg1 also downregulated the caspase-3 and caspase-9 activities in the diabetic hearts following myocardial I/R injury, but no difference was observed in the activities in the ginsenoside Re group.

Furthermore, the expression of apoptosis-related molecular markers was evaluated following the above treatment. Bcl-2 expression was observed via western blotting. The results showed that Bcl-2 expression in diabetic hearts was significantly reduced compared with that in the hearts of the control group, and after the administration of deferoxamine, Bcl-2 expression increased markedly (Fig. 4D). After application of ginsenoside Rg1, Bcl-2 expression also demonstrated a similar tendency as this observed for the HIF-1α activation group. The above effect was not observed in the Re treated group. Furthermore, Bax expression was also tested after the above treatment by western blotting. The results showed that Bax expression were

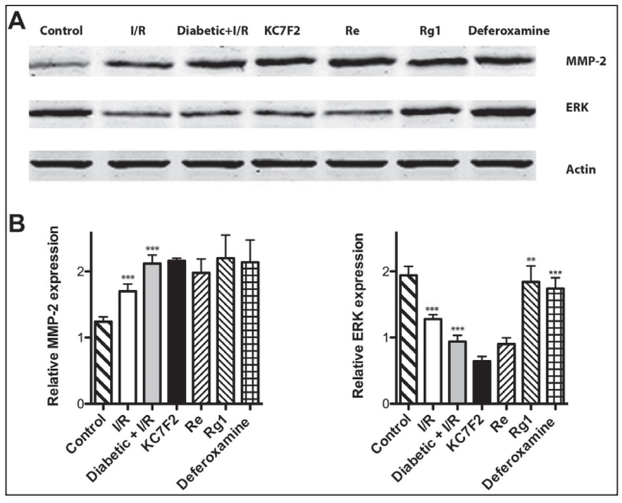

Fig. 5: Effect on ERK expression. (A) (B) MMP-2 and ERK levels were evaluated by Western blotting in diabetic rats after treatment with ginsenoside or deferoxamine. β-actin levels are shown as a loading control.

increased significantly both in I/R and in diabetic hearts, but after treatment with deferoxamine or Rg1, it was markedly reduced (Fig. 4D). The Bax/Bcl-2 ratio is shown in Fig. 4E.

2.4. Effect on ERK expression

To investigate possible downstream target molecules of HIF-1α involved in regulating myocardial ischaemia in diabetic rats, western blotting was performed to evaluate changes in protein expression following the above treatment (Fig. 5). The results showed that ERK expression in diabetic hearts was significantly reduced compared with that in the hearts of the control group, and after the administration of deferoxamine, ERK expression was markedly increased. There was no similar effect in the ginsenoside Re group, but ginsenoside Rg1 enhanced the ERK expression in diabetic rats. In addition, MMP-2 expression in diabetic hearts was also increased compared with that in the hearts of the control group and did not change by HIF-1α downregulation following the administration of deferoxamine or other chemicals.

3. Discussion

Reducing the high mortality associated with myocardial infarction following I/R in diabetes patients is an urgent issue, requiring the investigation of molecular mechanisms underlying this pathology and the identification of new therapeutic methods to protect the diabetic heart after I/R injury to enhance the survival and quality of life of these patients. Because of the importance of HIF-1α in diabetes and I/R injury, which has been demonstrated in previous studies (Wszola et al. 2014; Li et al. 2014), the application of a HIF-1α modulator has a high likelihood of showing therapeutic efficacy for regulating myocardial ischaemia in diabetic hearts.

The data obtained in the present work provide the following novel findings: (1) HIF-1α activation can effectively reduce myocardial injury following I/R in diabetic hearts; (2) HIF-1α activation can effectively inhibit apoptosis after myocardial injury following I/R in diabetic hearts; (3) the effect of HIF-1α activation on myocardial apoptosis in diabetic hearts is related to Bax/Bcl-2 expression as well as caspase-3 and caspase-9 activity; (4) the effect of HIF-1α activation on the regulation of myocardial ischaemia in diabetic rats occurs via ERK, but not MMP-2 signalling pathways; and (5) ginsenoside Rg1 application but not Re could generate the above similar effects via activating the HIF-1α-ERK pathway. Therefore, our data provided clear evidence that ginsenoside Rg1 may be the possible direct activator of HIF-1α, and that HIF-1α activation may reduce myocardial apoptosis and regulate ERK signalling pathways that are capable of modulating myocardial injury following I/R in diabetic hearts.

There is a higher risk of developing myocardial IR for diabetic patients than for non-diabetic subjects. Experimental data have been suggested that cardiac gene expression would accelerate the myocardial ischaemia and reperfusion injury due to diabetes (Benter et al. 2013; Di et al. 2018; Sivasinprasasn et al. 2017). In addition, blood glucose level was related with the myocardial ischaemia and reperfusion injury which could be affected by higher levels of proinflammatory cytokines in diabetic rat (Di et al. 2018; Kosuru et al. 2018; Sivasinprasasn et al. 2017). Our study provided the data that the blood glucose was reduced after administration of deferoxamine or ginsenoside Rg1.

It has been reported that CK, LDH, AST and ALT could be as the specific cardiac biomarkers and good indicators of myocardial injury, and serum activities of them will express the myocardial infarction level (Mythili et al. 2015; Zhu et al. 2015). The observed effects of HIF-1α activation on myocardial infarct size and plasma CK, LDH, AST and ALT levels in diabetic rats showed that HIF-1α activation could effectively reduce myocardial injury following I/R in diabetic hearts. Ginsenoside Rg1 application but not Re could activate HIF-1α to provide the above similar effect. The specific HIF-1α inhibitor KC7F2 could reduce the HIF-1α levels significantly (Cammarata et al. 2015; Cheng et al. 2016). In our study, there is no difference for above biomarkers between KC7F2 treatment group and diabetic rat model group. KC7F2 administration

could not change the myocardial ischaemia and reperfusion injury in a diabetic rat. Based on the data obtained in the present study, it is also clear that diabetes is an important risk factor in the magnitude of myocardial injury following I/R – a finding consistent with previous reports (Benter et al. 2013; Li et al. 2013). Therefore, additional experiments will be important for clarifying the mechanism underlying the effect of HIF-1α inhibition on infarct size.

Furthermore, myocardial apoptosis was monitored via TUNEL assays to evaluate the effect of HIF-1α activation on apoptosis in diabetic hearts. The results demonstrated that the percentage of TUNEL-positive myocyte nuclei was reduced following treatment with deferoxamine, which could specifically activate HIF-1α. The main risk factor for pathological processes of myocardial injury following ischaemia and reperfusion was myocardial apoptosis (Di et al. 2018; Bucciarelli et al. 2008). Thus, the prevention methods or therapeutic treatments that can inhibit cell apoptosis processes will reduce the level of myocardial injury. It has been demonstrated that HIF-1α activation positively regulates cell apoptosis (Abán et al. 2016; Mazelin et al. 2016; Azoitei et al. 2018), but few studies have invertigated the above effect of HIF-1α in myocardial injury following I/R, especially in diabetic hearts. Our current data provide direct evidence that HIF-1α activation by specific activators could reduce the myocardial apoptosis that enhances the myocardial injury. Therefore, it will benefit patients receiving therapy for clinicians and researchers to increase understanding of the underlying mechanism and find new therapeutic chemicals with higher efficacy and lower toxicity. Additionally, based on above results, ginsenoside Rg1 restricted apoptosis in diabetic hearts, and this effect may results from HIF-1α activation. As a potent activator, ginsenoside Rg1 is a good prospect for the prevention or treatment of the above disease.

Previous reports have demonstrated that Bax and Bcl-2 expression as well as caspase-3 and caspase-9 activities (apoptosis-related regulators in myocardial injury following I/R) play important roles during apoptosis progression (Feng et al. 2013; Ghosh et al. 2009; Rice et al. 2006). We therefore investigated changes in Bax and Bcl-2 expression and caspase-3 and caspase-9 activities. Our data provided evidence that diabetes was the cause of the observed enhancement of caspase-3 and caspase-9 levels after myocardial injury following I/R, consistent with previous studies (Chen et al. 2013; Li et al. 2008). Following the application of deferoxamine or ginsenoside Rg1, the reduction of caspase- 3 and caspase-9 activities, which can hamper apoptosis after myocardial injury following I/R, had a protective effect in diabetic hearts. The ratio of Bax / Bcl-2 expression also provided more details that myocardial ischaemia / reperfusion injury and diabetes are the important risk factors for myocardium apoptosis (Ghosh et al. 2009;Rice et al. 2006), and that the high level of myocardium apoptosis in diabetic hearts could be restricted by HIF-1α activation using a specific activator or ginsenoside Rg1.

Our results demonstrate that Bax, Bcl-2, caspase-3 and caspase-9 are the downstream regulators of HIF-1α in this pathological process. Activation of HIF-1α by ginsenoside Rg1 restricts the myocardial apoptosis and reduces the level of myocardial injury *via* apoptosis-related regulators. The details and possible pathway of the above process are still unknown but needed to explain the positive effect of ginsenoside Rg1.

It has been reported that ERK and MMP-2 play important roles in the development of pathologies in diabetic hearts, and it has been demonstrated that ERK is the direct regulator of the process causing myocardial injury following I/R (Itoh et al. 2012; Bhat et al. 2014). However, few studies have been performed to test whether ERK and MMP-2 are the downstream target molecules of HIF-1α in the regulation of myocardial ischaemia in diabetic rats. We therefore investigated this possibility in the current study to clarify the underlying mechanisms. The results obtained through western blotting provided direct evidence that ERK expression decreased after myocardial injury following I/R in diabetic hearts and that administration of a HIF-1α activator counteracts these effects but does not modulate MMP-2 expression. It is therefore clear that the effect of HIF-1α activation on myocardial ischaemia regulation in diabetic rats occurs *via* ERK, but not MMP-2 signalling pathways.

Here, we provide evidence that HIF-1α activation can effectively reduce myocardial injury following I/R in diabetic hearts through modulation of myocardial apoptosis related to Bax/Bcl-2 expression as well as caspase-3 and caspase-9 activities and that ginsenoside Rg1 but not Re is a potential activator of HIF-1α. We have also demonstrated that the observed protective effects are due to ERK but not MMP-2 signalling pathways. Our data demonstrate that HIF-1α is a potential molecular target for the design of therapeutic drugs for the protection of diabetic hearts after myocardial injury following I/R. In addition, ginsenoside Rg1 may be valuable in the above clinical therapeutic process. These findings provide useful information for developing new personalized therapeutic strategies for such clinical patients.

4. Experimental

4.1. Materials

Ginsenoside Rg1 and Re (Fig. 1) were obtained from Sigma-Aldrich (St. Louis, MO). In addition, specific inhibitor of HIF-1α including KC7F2 (Tocris, Bristol, UK) and specific activator of HIF-1α including deferoxamine (Sigma-Aldrich, St. Louis, MO) were used to modulate the effect of HIF-1α (Narita et al. 2009; Sorond et al. 2009).

4.2. Induction of diabetes

Rats were made diabetic by intravenous injection of streptozotocin (STZ) at a dose of 35 mg/kg (Yousif et al. 2012; Srinivasan et al. 2011). A glucometer (Johnson and Johnson, USA) was used to test the blood glucose concentration from tail vein blood after STZ treatment. When the blood glucose concentration reached 16.7 mmol/L, the diabetes rat model was validated (Koothappan et al. 2018; Ali et al. 2016).

4.3. Surgical preparations

Based on the previous report (Zheng et al. 2012), the rat model was created using the following procedures. First, the blood pressure was measured by inserting two cannulas into the left carotid artery and left femoral vein after anesthetization *via* sodium pentobarbital (30 mg/kg) administration. Furthermore, limb lead II of the ECG system was used to observe heart rate. To induce regional myocardial ischaemia, the left anterior descending coronary artery (LAD) was ligated with a silk suture 2 mm above the left auricle. The ligature was loosened to perform 2 h of reperfusion following 30 min of regional myocardial ischaemia. The rats in the control group were subjected to the same surgical procedures but without ligation of the LAD (Zheng et al. 2012).

4.4. Determination of infarct size

After the 2 h reperfusion, the left coronary artery was religated with the suture, and the right atrium was injected with 2 ml of 2% Evans blue via the left jugular vein to mark the ischaemic myocardium. Rat hearts were rapidly excised to remove the atria and the right ventricle. Slices were prepared as 2 mm-thick sections. The myocardial area at risk (AAR) and infarct area (IA) were measured in each slice via an image analysis system (Miki et al. 2009; Rodrigues et al. 2011).

4.5. Plasma enzyme assay

After reperfusion, arterial blood samples were collected and centrifuged at 3,000 rpm for 10 min. An analysis kit was used to measure creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the plasma for each group (Suanarunsawat et al. 2016; Suanarunsawat et al. 2010).

4.6. Determination of myocardial apoptosis

Myocardial apoptosis was tested in a terminal deoxynucleotidyl nick-end labelling (TUNEL) assay with an apoptosis detection kit (Roche, Basel, Switzerland). The percentage of TUNEL-positive cardiomyocytes in the ischaemic myocardium was observed in the following experiments to evaluate myocardial apoptosis levels (Bulani et al. 2017; Mokhtari et al. 2015). Caspase activities were also observed by the specific assay kit after the above application (Chatterjee et al. 2017; Lai et al. 2017; Li et al. 2016).

4.7. Western blotting

Protein expression was detected by western blotting after the above procedure. First, myocardium tissue samples were prepared in a buffer containing 0.1 M NaCl, 0.01 M Tris-HCl (pH 7.6), 1 mM EDTA (pH 8.0), 1 mg/ml aprotinin, 1% (w/v) Triton X-100, 100 mg/ml PMSF, 1% (w/v) NP-40, and 1 μg/ml leupeptin supplemented with proteinase and phosphatase inhibitor cocktails. Total protein was obtained from the samples after centrifugation at 12,000 × g for 60 min at 4 °C. The protein concentration in the lysates was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Proteins in the lysates were analysed through western blotting. Brifely, 30 μg of protein was separated via SDS-PAGE and electrophoretically transferred to a PVDF membrane. After blocking with 5% nonfat dry milk in PBST, the membrane

was incubated overnight at 4°C with primary antibodies against Bax, Bcl-2, ERK and MMP-2 (1:1000, Cell Signaling Technology, Beverly, MA, USA). Immunopositive bands were detected using the ECL-Plus enhanced chemiluminescence system. The blot was stripped and re-probed using an antibody against β-actin (Sigma-Aldrich, St. Louis, MO) to correct for any uneven loading or transfer of proteins.

Conflicts of interest: None declared.

References

Abán C, Martinez N, Carou C, Albamonte I, Toro A, Seyahian A, Franchi A, Leguizamón G, Trigubo D, Damiano A, Farina M (2016) Endocannabinoids participate in placental apoptosis induced by hypoxia inducible factor-1. Apoptosis 21:1094-1105.

Ali TM, Mehanna OM, Elsaid AG, Askary AE (2016) Effect of combination of angiotensin-converting enzyme inhibitors and vitamin D receptor activators on cardiac oxidative stress in diabetic rats. Am J Med Sci 352: 208-214.

Azoitei N, Becher A, Steinestel K, Rouhi A, Diepold K, Genze F, Simmet T, Seufferlein T (2016) PKM2 promotes tumor angiogenesis by regulating HIF-1α through NF-κB activation. Mol Cancer 15:3.

Benter IF, Babiker F, Al-Rashdan I, Yousif M, Akhtar S (2013) RU28318, an aldosterone antagonist, in combination with an ACE inhibitor and angiotensin receptor blocker attenuates cardiac dysfunction in diabetes. J Diabetes Res 2013: 427693.

Bhatt LK, Veeranjaneyulu A (2014) Enhancement of matrix metalloproteinase 2 and 9 inhibitory action of minocycline by aspirin: an approach to attenuate outcome of acute myocardial infarction in diabetes. Arch Med Res 45: 203-209.

Bohuslavova R, Kolar F, Sedmera D, Skvorova L, Papousek F, Neckar J, Pavlinkova G. (2014) Partial deficiency of HIF-1α stimulates pathological cardiac changes in streptozotocin-induced diabetic mice. BMC Endocr Disord 14: 11.

Bohuslavova R, Skvorova L, Sedmera D, Semenza GL, Pavlinkova G (2013) Increased susceptibility of HIF-1α heterozygous-null mice to cardiovascular malformations associated with maternal diabetes. J Mol Cell Cardiol 60: 129-141.

Brown NJ (2012) Cardiovascular effects of antidiabetic agents: focus on blood pressure effects of incretin-based therapies. J Am Soc Hypertens 6: 163-168.

Bucciarelli LG, Ananthakrishnan R, Hwang YC, Kaneko M, Song F, Sell DR, Strauch C, Monnier VM, Yan SF, Schmidt AM, Ramasamy M (2008) RAGE and modulation of ischemic injury in the diabetic myocardium. Diabetes 57: 1941-1951.

Bulani Y, Sharma SS (2017) Argatroban attenuates diabetic cardiomyopathy in rats by reducing fibrosis, inflammation, apoptosis, and protease-activated receptor expression. Cardiovasc Drugs Ther 31: 255-267.

Cammarata PR, Neelam S, Brooks MM (2015) Inhibition of hypoxia inducible factor-1α downregulates the expression of epithelial to mesenchymal transition early marker proteins without undermining cell survival in hypoxic lens epithelial cells. Mol Vis 21: 1024-1035.

Chatterjee B, Ghosh K, Yadav N, Kanade SR (2017) A novel L-fucose-binding lectin from Fenneropenaeus indicus induced cytotoxicity in breast cancer cells. J Biochem 161: 87-97.

Chen K, Li G, Geng F, Zhang Z, Li J, Yang M, Dong L, Gao F (2014) Berberine reduces ischemia/reperfusion-induced myocardial apoptosis via activating AMPK and PI3K-Akt signaling in diabetic rats. Apoptosis 19: 946-957.

Cheng CC, Guan SS, Yang HJ, Chang CC, Luo TY, Chang J, Ho AS (2016) Blocking heme oxygenase-1 by zinc protoporphyrin reduces tumor hypoxia-mediated VEGF release and inhibits tumor angiogenesis as a potential therapeutic agent against colorectal cancer. J Biomed Sci 23:18.

Das A, Durrant D, Koka S, Salloum FN, Xi L, Kukreja RC (2014) Mammalian target of rapamycin (mTOR) inhibition with rapamycin improves cardiac function in type 2 diabetic mice: potential role of attenuated oxidative stress and altered contractile protein expression. J Biol Chem 289: 4145-4160.

DeBerge M, Yeap XY, Dehn S, Zhang S, Grigoryeva L, Misener S, Procissi D, Zhou X, Lee DC, Muller WA, Luo X, Rothlin C, Tabas I, Thorp EB (2017) MerTK cleavage on resident cardiac macrophages compromises repair after myocardial ischemia reperfusion injury. Circ Res 121: 930-940.

Deng Y, Yang M, Xu F, Zhang Q, Zhao Q, Yu H, Li D, Zhang G, Lu A, Cho K, Teng F, Wu P, Wang L, Wu W, Liu X, Guo DA, Jiang B (2015) Combined salvianolic acid B and ginsenoside Rg1 exerts cardioprotection against ischemia/reperfusion injury in rats. PLoS One10: e0135435.

Di Paola R, Fusco R, Gugliandolo E, D'Amico R, Campolo M, Latteri S, Carughi A, Mandalari G, Cuzzocrea S (2018) The antioxidant activity of pistachios reduces cardiac tissue injury of acute ischemia/reperfusion (I/R) in diabetic streptozotocin (STZ)-induced hyperglycaemic rats. Front Pharmacol 9: 51.

Feng J, Liu Y, Dobrilovic N, Chu LM, Bianchi C, Singh AK, Sellke FW (2013) Altered apoptosis-related signaling after cardioplegic arrest in patients with uncontrolled type 2 diabetes mellitus. Circulation 128: S144-151.

Ghosh S, Khazaei M, Moien-Afshari F, Ang LS, Granville DJ, Verchere CB, Dunn SR, McCue P, Mizisin A, Sharma K, Laher I (2009) Moderate exercise attenuates caspase-3 activity, oxidative stress, and inhibits progression of diabetic renal disease in db/db mice. Am J Physiol Renal Physiol.296: F700-708.

Itoh T, Kouzu H, Miki T, Tanno M, Kuno A, Sato T, Sunaga D, Murase H, Miura T (2012) Cytoprotective regulation of the mitochondrial permeability transition pore is impaired in type 2 diabetic Goto-Kakizaki rat hearts. J Mol Cell Cardiol. 53: 870-879.

Kataoka Y, Yagi N, Kokubu N, Kasahara Y, Abe M, Otsuka Y (2011) Effect of pretreatment with pioglitazone on reperfusion injury in diabetic patients with acute myocardial infarction. Circ J 75: 1968-1974.

Kim JH, Yi YS, Kim MY, Cho JY (2017) Role of ginsenosides, the main active components of Panax ginseng, in inflammatory responses and diseases. J Ginseng Res 41: 435-443.

Koothappan M, Vellai RD, Subramanian IP, Subramanian SP (2018) Synthesis of a new zinc-mixed ligand complex and evaluation of its antidiabetic properties in high fat diet: low dose streptozotocin induced siabetic rats. Diabetes Metab J 42: 244-248.

Kosuru R, Cai Y, Kandula V, Yan D, Wang C, Zheng H, Li Y, Irwin MG, Singh S, Xia Z (2018) AMPK contributes to cardioprotective effects of pterostilbene against myocardial ischemia - reperfusion injury in diabetic rats by suppressing cardiac oxidative stress and apoptosis. Cell Physiol Biochem 46: 1381-1397.

Kravchuk E, Grineva E, Bairamov A, Galagudza M, Vlasov T (2011) The effect of metformin on the myocardial tolerance to ischemia-reperfusion injury in the rat model of diabetes mellitus type II. Exp Diabetes Res 2011: 907496.

Lai L, Chen J, Wang N, Zhu G, Duan X, Ling F (2017) MiRNA-30e mediated cardioprotection of ACE2 in rats with Doxorubicin-induced heart failure through inhibiting cardiomyocytes autophagy. Life Sci 169: 69-75.

Lejay A, Fang F, John R, Van JA, Barr M, Thaveau F, Chakfe N, Geny B, Scholey JW (2016) Ischemia reperfusion injury, ischemic conditioning and diabetes mellitus. J Mol Cell Cardiol 91: 11-22.

Li G, Lu WH, Ai R, Yang JH, Chen F, Tang ZZ (2014) The relationship between serum hypoxia-inducible factor 1α and coronary artery calcification in asymptomatic type 2 diabetic patients. Cardiovasc Diabetol 13: 52.

Li H, Bian Y, Zhang N, Guo J, Wang C, Lau WB, Xiao C (2013) Intermedin protects against myocardial ischemia-reperfusion injury in diabetic rats. Cardiovasc Diabetol 12: 91.

Li J, Lang MJ, Mao XB, Tian L, Feng YB (2008) Antiapoptosis and mitochondrial effect of pioglitazone preconditioning in the ischemic/reperfused heart of rat. Cardiovasc Drugs Ther 22: 283-291.

Li YH, Fu HL, Tian ML, Wang YQ, Chen W, Cai LL, Zhou XH, Yuan HB (2016) Neuron-derived FGF10 ameliorates cerebral ischemia injury via inhibiting NF-κB-dependent neuroinflammation and activating PI3K/Akt survival signaling pathway in mice. Sci Rep 6: 19869.

Lu, M, Yao, Q, Chen, C (2009) Ginseng compounds: an update on their molecular mechanisms and medical applications. Curr Vasc Pharmacol 7: 293–302.

Mazelin L, Panthu B, Nicot AS, Belotti E, Tintignac L, Teixeira G, Zhang Q, Risson V, Baas D, Delaune E, Derumeaux G, Taillandier D, Ohlmann T, Ovize M, Gangloff YG, Schaeffer L (2016) mTOR inactivation in myocardium from infant mice rapidly leads to dilated cardiomyopathy due to translation defects and p53/JNK-mediated apoptosis. J Mol Cell Cardiol 97: 213-225.

Messaoudi S, Rongen GA, Riksen NP (2013) Metformin therapy in diabetes: the role of cardioprotection. Curr Atheroscler Rep 15: 314.

Miki T, Itoh T, Sunaga D, Miura T (2012) Effects of diabetes on myocardial infarct size and cardioprotection by preconditioning and postconditioning. Cardiovasc Diabetol 11: 67.

Miki T, Miura T, Hotta H, Tanno M, Yano T, Sato T, Terashima Y, Takada A, Ishikawa S, Shimamoto K (2009) Endoplasmic reticulum stress in diabetic hearts abolishes erythropoietin-induced myocardial protection by impairment of phospho-glycogen synthase kinase-3beta-mediated suppression of mitochondrial permeability transition. Diabetes 58: 2863-2872.

Miki T, Yuda S, Kouzu H, Miura T (2013) Diabetic cardiomyopathy: pathophysiology and clinical features. Heart Fail Rev 18: 149-166.

Miswan Z, Lukman SK, Abd Majid FA, Loke MF, Saidin S, Hermawan H (2016) Drug-eluting coating of ginsenoside Rg1 and Re incorporated poly(lactic-co-glycolic acid) on stainless steel 316L: Physicochemical and drug release analyses. Int J Pharm 515: 460-466.

Mokhtari B, Badalzadeh R, Alihemmati A, Mohammadi M (2015) Phosphorylation of GSK-3β and reduction of apoptosis as targets of troxerutin effect on reperfusion injury of diabetic myocardium. Eur J Pharmacol 765: 316-321.

Mythili S, Malathi N (2015) Diagnostic markers of acute myocardial infarction. Biomed Rep 3: 743-748.

Narita T, Yin S, Gelin CF, Moreno CS, Yepes M, Nicolaou KC, Van Meir EG (2009) Identification of a novel small molecule HIF-1alpha translation inhibitor. Clin Cancer Res 15: 6128-6136.

Nishimoto A, Kugimiya N, Hosoyama T, Enoki T, Li TS, Hamano K (2014) HIF-1α activation under glucose deprivation plays a central role in the acquisition of anti-apoptosis in human colon cancer cells. Int J Oncol 44: 2077-2084.

Radad K, Gille G, Moldzio R, Saito H, Rausch D (2004) Ginsenosides Rb1 and Rg1 effects on mesencephalic dopaminergic cells stressed with glutamate. Brain Res 1021: 41–53.

Rice KM, Desai DH, Kakarla SK, Katta A, Preston DL, Wehner P, Blough ER (2006) Diabetes alters vascular mechanotransduction: pressure-induced regulation of mitogen activated protein kinases in the rat inferior vena cava. Cardiovasc Diabetol 5: 18.

Rodrigues B, Rosa KT, Medeiros A, Schaan BD, Brum PC, De Angelis K, Irigoyen MC (2011) Hyperglycemia can delay left ventricular dysfunction but not autonomic damag after myocardial infarction in rodents. Cardiovasc Diabetol 10: 26.

Sen Banerjee S, Thirunavukkarasu M, Tipu Rishi M, Sanchez JA, Maulik N, Maulik G (2013) HIF-prolyl hydroxylases and cardiovascular diseases. Toxicol Mech Meth 22: 347-358.

Sivasinprasasn S, Tanajak P, Pongkan W, Pratchayasakul W, Chattipakorn SC, Chattipakorn N (2017) DPP-4 inhibitor and estrogen share similar efficacy against cardiac ischemic-reperfusion injury in obese-insulin resistant and estrogen-deprived female rats. Sci Rep 7: 44306.

Sorond FA, Shaffer ML, Kung AL, Lipsitz LA (2009) Desferroxamine infusion increases cerebral blood flow: a potential association with hypoxia-inducible factor-1. Clin Sci (Lond) 116: 771-779.

Srinivasan K, Sharma SS (2011) Augmentation of endoplasmic reticulum stress in cerebral ischemia/reperfusion injury associated with comorbid type 2 diabetes. Neurol Res 33: 858-865.

Suanarunsawat T, Anantasomboon G, Piewbang C (2016) Anti-diabetic and anti-oxidative activity of fixed oil extracted from Ocimum sanctum L. leaves in diabetic rats. Exp Ther Med 11: 832-840.

Suanarunsawat T, Devakul Na Ayutthaya W, Songsak T, Thirawarapan S, Poungshompoo S (2010) Antioxidant activity and lipid-lowering effect of essential oils extracted from Ocimum sanctum L. leaves in rats fed with a high cholesterol diet. J Clin Biochem Nutr 46: 52-59.

Tao L, Gao E, Jiao X, Yuan Y, Li S, Christopher TA, Lopez BL, Koch W, Chan L, Goldstein BJ, Ma XL (2007) Adiponectin cardioprotection after myocardial ischemia/reperfusion involves the reduction of oxidative/nitrative stress. Circulation 115: 1408-1416.

Wszola M, Kwiatkowski A, Domagala P, Wirkowska A, Bieniasz M, Diuwe P, Rafał K, Durlik M, Chmura A (2014) Preservation of kidneys by machine perfusion influences gene expression and may limit ischemia/reperfusion injury. Prog Transplant 24: 19-26.

Yin X, Zheng Y, Zhai X, Zhao X, Cai L (2012) Diabetic inhibition of preconditioning- and postconditioning-mediated myocardial protection against ischemia/reperfusion injury. Exp Diabetes Res 2012: 198048.

Yousif MH, Dhaunsi GS, Makki BM, Qabazard BA, Akhtar S, Benter IF (2012) Characterization of angiotensin-(1-7) effects on the cardiovascular system in an experimental model of type-1 diabetes. Pharmacol Res 66: 269-275.

Zhang ZL, Fan Y, Liu ML (2012) Ginsenoside Rg1 inhibits autophagy in H9c2 cardiomyocytes exposed to hypoxia/reoxygenation. Mol Cell Biochem 365: 243-250.

Zheng LR, Han J, Yao L, Sun YL, Jiang DM, Hu SJ, Shao L, Sun ZH, Wang LH (2012) Up-regulation of calcitonin gene-related peptide protects streptozotocin-induced diabetic hearts from ischemia/reperfusion injury. Int J Cardiol 156: 192-198.

Zhu Y, Zhang H, Sun Y, Li Y, Deng L, Wen X, Wang H, Zhang C (2015) Serum enzyme profiles differentiate five types of muscular dystrophy. Dis Markers 2015: 543282.