Preventive Effects of Exenatide on Endothelial Dysfunction Induced by Ischemia-Reperfusion Injury via K_{ATP} Channels

Sang Jin Ha, Weon Kim, Jong Shin Woo, Jee Tae Kim, Soo Joong Kim, Woo-Shik Kim, Myeong Kon Kim, Xian Wu Cheng, Kwon Sam Kim

Objective—The purpose of this study was to evaluate whether exenatide administration can prevent impairment in endothelium-dependent vasodilation induced by ischemia-reperfusion (IR) injury and whether this effect is mediated by K_{ATP} channel opening.

Methods and Results—In a double-blind, placebo-controlled, crossover design, 20 volunteers were randomly assigned to 2 groups: subcutaneous exenatide (10 μg) or placebo administration. At 30 minutes after the study drug administration, endothelium-dependent flow-mediated dilatation (FMD) of the radial artery was measured before and after IR (15 minutes of ischemia at the level of the brachial artery followed by 15 minutes of reperfusion) injury. Seven days later, both groups were crossed over and received the other treatment (ie, placebo or exenatide) and underwent the same protocol. Pre-IR radial artery diameter, FMD, and baseline radial artery diameter after IR injury were similar between 2 groups (P=no significant difference). After placebo administration, IR significantly blunted FMD (before IR: 12.0±6.23%; after IR: 4.6±3.57%; P=0.02). Exenatide prevented this impairment (FMD before IR: 15.0±7.14%; FMD after IR: 15.0±5.96%; P=no significant difference; P<0.001 compared with placebo). In a separate protocol, this protective effect was completely abolished by pretreatment with glibenclamide (glyburide, 5 mg), a blocker of K_{ATP} channels (n=7; FMD before IR: 12.0±2.2%; after IR: 3.2±2.1%, P<0.001).

Conclusion—The present study demonstrates that subcutaneous exenatide protects IR-induced endothelial dysfunction through opening of K_{ATP} channels in human IR injury model. (Arterioscler Thromb Vasc Biol. 2012;32:474-480.)

Key Words: endothelium ■ ischemia ■ pharmacology ■ reperfusion injury

Previous studies have emphasized the importance of the vascular endothelium in the pathophysiology of tissue injury induced by ischemia-reperfusion (IR).1 Endothelial cells seem to be more prone to IR injury than cardiac myocytes, and during ischemia the appearance of IR-induced tissue necrosis is temporally preceded by a state of reduced endothelial responsiveness to specific stimuli (endothelial dysfunction).2,3 Many studies have demonstrated that preexposure to brief periods of ischemia (ie, ischemic preconditioning) or specific pharmacological stimuli (pharmacological preconditioning) can adjust myocardial sensitivity to IR-induced injury.4 Furthermore, multiple lines of evidence suggest that stimuli leading to K_{ATP} channel activation and opening can induce a potent protective effect against IR in different cell types. For example, animal models have documented that the administration of specific openers of K_{ATP} channels leads to a state of reduced susceptibility to IR injury in the endothelium, whereas blockades of these channels with sulfonylureas inhibit endothelial cell ischemic preconditioning.5 Gori et al6 showed that oral sildenafil induces a protection against IR-induced endothelial dysfunction through opening of K_{ATP} channels and is the first pharmacological preconditioning agent in human.

Exenatide (exendin-4) is a 39–amino acid peptide originally derived from the saliva of the gila monster, a venomous lizard native to the southwestern United States and northern Mexico. Exenatide mimics human glucagon-like peptide (GLP)-1, a gut incretin hormone that is released in response to nutrient intake.7 It exerts insulinotropic and insulinominetic properties via the G-protein–coupled GLP-1 receptor, which has also been reported to be expressed in the heart.8,9 The GLP-1 receptor has been shown to be cardioprotective in a rat model of myocardial IR injury.10 Timmers et al11 demonstrated compelling evidence that exenatide confers strong cardioprotection and improves left ventricular systolic and diastolic functions in a clinically relevant large animal model of IR injury. However, there was little evidence of the effect of exenatide on the endothelium, especially IR-induced endothelial dysfunction.

In a human in vivo model of IR-induced endothelial dysfunction, we designed the protocols to test (1) whether exenatide administration can prevent impairment in endothelium-dependent vasodilation, (2) whether exenatide prevents IR-induced endothelial dysfunction, and (3) whether this effect is mediated by K_{ATP} channel opening.
lum-dependent vasodilation induced by IR injury and (2) whether this effect is mediated by $K_{\text{ATP}}$ channel opening.

Materials and Methods

The ethics committee of the Kyung Hee University approved this study, and written informed consent was obtained in all cases. Studies were conducted in a quiet, temperature- and humidity-controlled environment.

Protocol 1: The Effect of Exenatide on IR Injury in the Endothelium

Twenty healthy nonsmoking volunteers (25–40 years old) were enrolled in this double-blind, randomized, placebo-controlled crossover trial. All subjects were requested to abstain from caffeine or any drugs, including supplemental vitamins, for the duration of the study. All subjects were randomly assigned to receive placebo or 10 $\mu$g of exenatide by subcutaneous injection (Byetta, Lilly). Ten subjects had received the placebo as the first treatment, whereas the other 10 had initially received exenatide. With study drugs administration, a 10% dextrose infusion was started and was titrated to maintain blood sugar levels between 80 and 120 mg/dL throughout the study period. Initially, radial artery endothelium-dependent flow-mediated dilatation (FMD) and endothelial-independent nitroglycerin-mediated dilatation (NMD) were measured as described in detail below. Subsequently, 1.5 hours later, IR injury was performed by a maneuver which placed a pneumatic cuff at the level of the brachial artery and inflated to 200 mm Hg for 15 minutes to induce radial artery ischemia. At the end of ischemia, 15 minutes of repertusion was performed to induce repertusion injury. After IR injury, radial artery FMD was measured again (Supplemental Figure I, available online at http://atvb.ahajournals.org). All volunteers had a wash-out period of 7 days. Seven days later, the subjects returned to crossover study medication (ie, exenatide or placebo), and the protocol described above was repeated.

Blood samples (for insulin analysis) and supine blood pressure were taken to record at 3 points (before examination, after baseline FMD and NMD, and after the end of examination) in protocol 1. Plasma insulin levels were assessed by ELISA (BenderMed Systems).

Protocol 2: Effect of Glibenclamide

In a separate protocol, 7 healthy volunteers were administered 5 mg of glibenclamide (glyburide, Euglucon, Roche Pharma) 1 hour before administration of 10 $\mu$g of exenatide. This dosage has previously been shown to be able to completely inhibit forearm $K_{\text{ATP}}$ channels.9 With the glibenclamide administration, a 10% dextrose infusion was started and was titrated to maintain blood sugar levels between 80 and 120 mg/dL throughout the study period. One and a half hours after exenatide administration, the subjects underwent FMD measurement before and after IR, as described above. Because of safety concerns related to the potent hypoglycemic effect of glibenclamide requiring continuous adjustment of a dextrose infusion, this protocol was not double-blind. All subjects except for 1 also participated in protocol 1.

Measurement of Radial Artery Diameter and FMD

Subjects were asked to rest for 10 minutes in the supine position before each study protocol. The FMD and NMD measurements were performed as follows by 2 independent operators. Radial artery images were performed using commercially available system (Vivid 7, GE Vingmed, Horten, Norway) equipped with a 14-MHz linear array transducer. The ECG-gated, end-diastolic, longitudinal, B-mode images were digitally stored on the hard disk of the instrument for online and offline analysis. For FMD measurement, the baseline radial artery diameter was averaged from 6 separate images taken at 5-second intervals. Subsequently, a pneumatic cuff placed at the level of the wrist (ie, distal to the site of radial artery measurement) was inflated to 250 mm Hg for 5 minutes. After wrist-cuff deflation, radial artery diameter was reexamined and averaged from 6 separate images taken at 5-second intervals. FMD was calculated as the percentage maximum increase in arterial diameter. After a 10-minute rest period to allow restoration of baseline conditions, NMD was assessed by obtaining 2-dimensional images before and 3 minutes after administration of 50 $\mu$g of GTN for determining endothelial independent vasodilation.9 Radial artery diameter was calculated from the trailing edge of the intima-blood interface to the leading edge semiautomatically using a modified version of ImageJ software (National Institutes of Health), as well as custom-designed software. Baseline and post-IR (reactive hyperemia) radial blood flows were measured using pulsed-wave Doppler as an average velocity-time integral for the first 5 cardiac cycles after cuff deflation and were multiplied by heart rate and vessel cross-sectional area.

A flow chart illustrating the measurement protocol and time points is shown in Supplemental Figure I.

Statistical Analysis

All of the data are presented as the mean±standard deviation. Baseline values were compared by use of a paired $t$ test (between visits, protocol 1) or an unpaired $t$ test (between protocols). The effect of IR on radial artery diameters, reactive hyperemia, and FMD within each study visit (protocols 1 and 2) was tested by use of a paired $t$ test. One-way ANOVA (comparison of 3 or more groups for FMD) followed by the Tukey post hoc test was used for statistical analysis. Between-group differences (exenatide versus placebo and pre-IR versus post-IR) of artery diameter and blood flow in protocol 1 were analyzed by use of a 2-way ANOVA for repeated measures. A value of $P<0.05$ was set as the threshold for significance. The analyses were performed using software (SPSS version 17.0, SPSS, Chicago, IL).

Results

The radial artery diameter and blood flow measurements are presented in Tables 1 and 2, and FMD data are reported in Figures 1 and 2. To confirm interobserver and intraobserver correlations for reproducibility, we performed measurements of the same data by 2 other people and repeated the measurements 3 months later. There was good correlation between the interobserver and intraobserver coefficients ($R>0.9$).

Protocol 1

Effects of Exenatide Administration on the Baseline Parameters

Exenatide administration had no effect on arterial blood pressure (placebo 113±5 mm Hg versus exenatide 116±7 mm Hg; placebo 77±7 mm Hg versus exenatide 75±16 mm Hg; $P=$no significant difference [NS]). Also, exenatide had no effect on radial artery diameter, FMD, or NMD before IR (Table 1 and Figure 1, $P=$NS). However, exenatide had an effect on blood flow as expressed in reactive hyperemia, shown in Table 2 (50.7±57.2% versus 210±120%, $P<0.001$).

Effects of IR After Placebo Administration

In the placebo group, radial artery diameter and blood flow values were not different from baseline values before and after IR ($P=$NS). But post-IR FMD was significantly reduced when it was compared with the pre-IR level (before IR: 12.0±6.23%; after IR: 4.6±3.57%, $P=0.02$, Table 1 and Figure 1). Also, decreased reactive hyperemia was noted (before IR: 50.7±57.2%; after IR: 23.6±32.3%, $P<0.05$, Table 2) but no significant change in NMD was observed.
effects of IR injury (P<0.05 compared with placebo after IR; 2-way ANOVA) without effect on NMD. During the protocol, insulin level at the point of first FMD measurement and end of examination increased significantly and gradually compared with baseline insulin level before examination (3.6±3.5 versus 22.0±4.9 versus 34.3±5.8 µU/mL, P<0.05). However, there was no statistical difference in the blood sugar levels among baseline, 1st FMD measurement, and end of examination (90±5.7 versus 85±3.5 versus 82±2.3 mg/dL, P=NS). Exenatide had

Table 2. Results of Blood Flow of the Radial Artery in Protocols 1 and 2

<table>
<thead>
<tr>
<th>Blood Flow</th>
<th>Artery Diameter Pre-IR</th>
<th>Artery Diameter Post-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artery Diameter Pre-IR</td>
<td>Artery Diameter Post-IR</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Hyperemia</td>
</tr>
<tr>
<td></td>
<td>Blood Flow (mL/min)</td>
<td>After Wrist Cuff</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>Deflation (mm)</td>
</tr>
<tr>
<td>FMD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>12.0±6.23</td>
<td>2.47±0.33</td>
</tr>
<tr>
<td>Exenatide</td>
<td>15.0±7.14</td>
<td>2.44±0.41</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilben+exenatide</td>
<td>12.02±2.16</td>
<td>2.44±0.44</td>
</tr>
<tr>
<td>NMD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>10.4±3.24</td>
<td>2.51±0.22</td>
</tr>
<tr>
<td>Exenatide</td>
<td>10.8±0.42</td>
<td>2.49±0.34</td>
</tr>
</tbody>
</table>

Values are mean±standard deviation. FMD indicates flow-mediated dilatation; NMD, nitroglycerin-mediated dilatation; IR, ischemia-reperfusion.

*P<0.05 vs corresponding controls (before IR).
†P<0.05 vs corresponding controls (placebo in protocol 1).
‡P<0.05 vs corresponding controls (baseline blood flow).
§P<0.05 vs corresponding controls (pre-IR).

Effects of IR After Exenatide Administration
In the exenatide group, radial artery was dilated after IR injury (P=NS compared with exenatide before IR and with placebo after IR). IR significantly affected the peak reactive hyperemia despite of exenatide administration (before IR: 210±120%; after IR: 55.3±32.5%, P<0.001 compared with before IR, Table 2), but there was no statistical difference in NMD between the placebo and the exenatide groups (P=NS, Table 2). Although IR injury significantly reduced blood

Figure 1. Box plots of the flow-mediated dilatation (FMD) responses before and after ischemia-reperfusion (IR) (Protocol 1). Left: In the placebo group, FMD was significantly blunted after IR. Right: Exenatide completely prevented this impairment in endothelium-dependent vasodilation induced by IR (Tukey-type multiple comparison test). Boxes show interquartile ranges; the lower and upper boundaries of the boxes indicate the 25th and 75th percentile levels, respectively; and the horizontal lines within the boxes indicate the median levels.
Figure 2. Box plots of the flow-mediated dilatation (FMD) responses before and after ischemia-reperfusion (IR) following administration of glibenclamide and exenatide (protocol 2). FMD was significantly blunted after IR, demonstrating that glibenclamide inhibited exenatide-induced endothelial protection against IR. Boxes show interquartile ranges; the lower and upper boundaries of the boxes indicate the 25th and 75th percentile levels, respectively; and the horizontal lines within the boxes indicate the median levels.

no effect on radial artery diameter, FMD, or NMD before IR (Table 1 and Figure 1, P=NS).

Protocol 2

Effect of Glibenclamide
Radial artery diameter and blood flow data are reported in Tables 1 and 2. Although reactive hyperemia significantly different (exenatide only: 210±120%; glibenclamide+exenatide: 51±28%, P<0.001, Table 2), pre-IR baseline radial artery diameter and FMD were not different after glibenclamide plus exenatide compared with exenatide alone. After IR, baseline radial artery diameter returned to the values observed before IR. However, despite the greater blood flow stimulus caused by the exenatide, FMD was blunted to much lower values than those observed in the placebo group of protocol 1, demonstrating a potent inhibitory effect of K\textsubscript{ATP} channel blockade on exenatide-induced endothelial protection (exenatide only: 12.0±2.2%; glibenclamide+exenatide: 3.2±2.1%, P<0.001 compared with before IR, Figure 2). In addition, this blunting effect of glibenclamide overcame the increased blood flow effect of exenatide (Table 2). These results demonstrated that exenatide is K\textsubscript{ATP} channel dependent (glibenclamide sensitive). During protocol 2, no hypoglycemic events developed, and blood sugar level was maintained (before protocol 1: 97.0±3.5 mg/dL; end of protocol: 82±2.3 mg/dL).

Discussion
This study showed that exposure to IR impaired FMD at the level of the forearm conductance vessels and that impaired FMD caused by IR can be prevented by pretreatment with exenatide, which mimics ischemic preconditioning at the endothelium level; this effect is blunted by the K\textsubscript{ATP} channel blocker glibenclamide. To the best of our knowledge, this is the first report demonstrating that exenatide induces potent protection against IR-induced endothelial dysfunction through the opening of K\textsubscript{ATP} channels.

Conduit arteries (eg, the brachial, radial, femoral, or popliteal) dilate in response to an increase in blood flow.\textsuperscript{12–14} This physiological response is dependent on the presence of an intact endothelium,\textsuperscript{15,16} and the measurement of FMD in vivo has been widely adopted as an assessment of endothelial function.\textsuperscript{17} Peripheral artery disease, a manifestation of atherosclerosis, is a very common comorbidity seen in the setting of coronary artery disease because the 2 conditions share a similar pathogenesis, including endothelial dysfunction.\textsuperscript{18,19} It is well known that the examination of FMD in peripheral artery is the most widely used noninvasive ultrasound method to assess endothelial function.\textsuperscript{20,21} Previously, Kuvik et al\textsuperscript{22} reported that peripheral vascular endothelial function testing as noninvasive indicator of coronary artery disease. Furthermore, it has recently been reported that impaired FMD is the only significant factor associated with acute ST-elevation myocardial infarction.\textsuperscript{23} It seems to be that the standard FMD model used here could be applied to not only evaluate peripheral but also coronary artery endothelial dysfunction.

The results of cardiac IR injury are not restricted to myocytes but also affect the coronary endothelium, where they are characterized by decreased nitric oxide (NO)–dependent relaxations. Given the key role of the endothelium and NO in the control of vascular tone, as well as platelet and leukocyte functions, protection of coronary endothelial cells is an important therapeutic target in IR injury.\textsuperscript{24,25} The pathophysiological significance of reperfusion-induced coronary endothelial injury of large coronary arteries may be related, at least in part, to the properties of endothelium- derived NO at this level. Indeed, constitutive NO production continuously opposes vasoconstrictor influences in large coronary arteries.\textsuperscript{26} Moreover, NO has an inhibitory effect on platelet aggregation and leukocyte activation and adhesion.\textsuperscript{27,28} Thus, endothelial dysfunction may lead to vasoconstriction and increased platelet aggregation, resulting in subsequent increased risks of vasospasm and thrombosis, and NO has the protective effects of preconditioning against reperfusion development of atherosclerosis.\textsuperscript{29,30}

In a previous report, Kharbanda et al\textsuperscript{3} demonstrated that a clinically relevant period of IR causes profound and sustained endothelial dysfunction assessed by FMD at the level of the forearm conductance vessels while leaving endothelium-independent vasodilation unchanged. In their report, exposure to brief episodes of ischemia (ie, ischemic preconditioning) attenuates this specific impairment in endothelium-dependent relaxation. Animal studies have demonstrated that administration of pharmacological stimuli, such as adenosine, bradykinin, nitric oxide donors, and opioids, can induce a protective phenomenon analogous to ischemic preconditioning.\textsuperscript{29} Importantly, a key stage in the complicated molecular pathways activated by these mediators, as well as by ischemic preconditioning, seems to be the opening of K\textsubscript{ATP} channels. As a result, mitochondrial K\textsubscript{ATP} opening evokes a response involving several different protective mechanisms, including matrix swelling, reactive oxygen species modulation, and
effects on mitochondrial Ca\(^{2+}\) homeostasis.\(^{5,30,31}\) Interestingly, multiple lines of evidence have emphasized the role of endogenous (endothelial) and exogenous nitric oxide in the physiologies of both ischemic and pharmacological preconditionings\(^{32}\) directly and via opening of K\(_{\text{ATP}}\) channels.\(^{30,33}\) This pathway might have clinical relevance, because (1) administration of a nitric oxide donor can reduce myocardial ischemia during angioplasty,\(^{34}\) and (2) administration of K\(_{\text{ATP}}\) channel blocker suramin in diabetic patients.\(^{43}\) Furthermore, GLP-1 infusion enhanced blood pressure lowering effect and could be applied to reperfusion.\(^{37}\) Experiments in both isolated and in vivo rat preparations directly and via opening of K\(_{\text{ATP}}\) channels.\(^{30,33}\)

Downey et al\(^{36}\) have developed the hypothesis that stimulation of a variety of G protein–coupled receptors results in the activation of protein kinase C. This, in turn, leads to the translocation of protein kinase C from the cytoplasm to the sarcolemma, where it phosphorylates a substrate protein (possibly the ATP-sensitive K1 [K\(_{\text{ATP}}\)] channel), which confers resistance to ischemia.

Exenatide/exendin-4 and GLP-1 are known to have a number of cardiovascular effects.\(^{11,37,38}\) In isolated perfused rat hearts, exendin-4 reduced infarct size when administered at reperfusion.\(^{37}\) Experiments in both isolated and in vivo rat hearts suggest that GLP-1 significantly attenuates reperfusion injury and reduces infarct size by approximately 50% when coadministered with a dipeptidyl peptidase-IV inhibitor.\(^{10}\) In recent research, Timmers et al\(^{11}\) presented data in which the incretin mimetic exenatide significantly reduces myocardial infarction and improves left ventricular contraction and relaxation when it is given at reperfusion. Emerging lines of evidence show an additional benefit of GLP-1 on the endothelium. Indeed, except for cardiomyocytes, GLP-1R expression has been detected on endothelial and vascular smooth muscle cells, as well as on macrophages and monocytes.\(^{39}\) Ban et al\(^{40}\) proposed a novel 2-pathway schema for cardio-vascular actions of GLP-1: in 1 pathway, binding to the GLP-1 receptor mediates effects on cardiac inotropic action, glucose uptake, ischemic preconditioning and mild vasodilatation; the other pathway depends on the rapid breakdown of GLP-1 to GLP-1(9–36) and the consequent receptor-independent effects on the postischemic recovery of cardiac function and vasodilatation. GLP-1(9–36) also appeared to improve the survival of human aortic endothelial cells after IR.\(^{40}\) These actions were also exerted through the nitric oxide synthase pathway.\(^{38}\) Nevertheless, some investigators observed a vasodilatory effect of GLP-1 independently of NO, indicating clearly a direct action on vascular smooth muscle cells via its GLP-1R.\(^{41}\) In particular, in type 2 diabetes mellitus patients with coronary artery disease, rGLP-1 infusions (at a dose of 2 pmol/kg per minute) significantly increased FMD in the brachial artery compared with placebo, but in healthy subjects, GLP-1 infusion did not affect the FMD.\(^{42}\) Recent studies demonstrated that exenatide had the blood pressure lowering effect and could be applied to diabetes patients.\(^{43}\) Furthermore, GLP-1 infusion enhanced acetylcholine-mediated vasodilation in nondiabetic, normotensive nonsmokers, an effect that was abolished after coadministration of glyburide (but not glimepiride). These data indicate also a potential modulatory role of sulfonylurea receptor subunit on GLP-1Rs in the endothelial cells and a selectivity of K\(_{\text{ATP}}\) channel inhibition among different sulfonylurea agents.\(^{44}\)

In our study, exenatide (or the combination of exenatide and glibenclamide) had no effect on baseline radial artery diameter, NMD, and FMD but increased the blood flow, expressed as reactive hyperemia. In the point of the view that the reactive hyperemia is the stimulus for FMD, it is likely that there was discrepancy in the FMD and reactive hyperemia data. Further study will be required to investigate for this issue. Additionally, it has been reported that GLP-1 infusion had no affect on FMD and nitroglycerin responses in the healthy subjects, whereas it improved endothelial dysfunction in type 2 diabetes mellitus with coronary heart disease.\(^{42}\) As in the our protocol dealing with healthy subjects, there was no effect of GLP-1 pretreatment on FMD and NMD-mediated response. On the contrary, the endothelial dysfunction induced by IR injury was not observed in the exenatide pretreatment group. These findings suggested that the pretreatment with exenatide prevented endothelial dysfunction induced by IR injury.

The effect of GLP-1 on blood flow can be explained by a previous animal experiment showing that GLP-1 induces potent relaxation of rat conduit arteries via a specific receptor independently of NO and the endothelium.\(^{41}\) Previous studies have shown that the IR injury used here specifically impairs endothelium-dependent response while leaving endothelium-independent reactivity unaltered.\(^{3}\) Therefore, the fact that reactive hyperemia, a predominantly NO-independent process,\(^{45}\) is unimpaired in the present study should be expected. However, radial artery FMD after IR was significantly reduced in the placebo group, whereas the administration of exenatide remarkably limited this effect. These data demonstrate that although IR is able to induce a significant impairment in endothelium-dependent vasodilatation, pretreatment with exenatide can protect the endothelium from IR injury via a specific molecular pathway. As in our data from protocol 2, the endothelial protective effect of exenatide was almost completely prevented when a K\(_{\text{ATP}}\) channel blocker was administered before the exenatide. Although hyperglycemia induces oxidative and inflammatory stress and suppresses endothelial nitric oxide synthase, we maintained the normoglycemic level throughout the experiment. This demonstrated that exenatide is K\(_{\text{ATP}}\) channel-dependent (glibenclamide-sensitive) and it is likely that the protective effect of exenatide is mediated by K\(_{\text{ATP}}\) channel opening. Overall, the results suggest that exenatide exerts the ischemic preconditioning effect through the NO-dependent pathway, of which K\(_{\text{ATP}}\) channels are a key effector molecule. It should be noted that 1 likely explanation for the higher FMD of IR with exenatide is a difference in the flow stimulus, as this was statically higher and is the stimulus for dilation. This suggests that an NO-independent effect may be responsible for exenatide-mediated higher FMD after IR.

With the exenatide and glibenclamide administration, a 10% dextrose infusion was maintained and was titrated to maintain blood sugar levels between 80 and 120 mg/dL throughout the study period. As we are well aware, the action of the GLP-1 analog exenatide increases insulin secretion from \(\beta\)-cells via glucose-dependent stimulation of insulin
secretion mechanism. The half-life of exenatide is 2.4 hours, and it raises insulin levels quickly (within \(\approx 10\) minutes of administration), with the insulin levels subsiding substantially over the next hour or 2. In our data, insulin levels gradually increased at the end of measurement protocol, 55 minutes after exenatide injection.

Our data were collected from forearm blood circulation in healthy volunteers, so direct comparison with coronary circulation has some limitations. The study model does not show the specific molecular mechanisms involved in the protective effect. The exact cellular and subcellular sites on which exenatide and glibenclamide act, as well as the exact mediators involved, will have to be investigated with in vitro studies. However, because previous studies have demonstrated that the IR protocol used here does not affect endothelium-independent vascular responses and because of the evidence that endothelial cells, like smooth muscle cells, possess the apparatus necessary to develop preconditioning, we propose a direct involvement of endothelial cells in our model. In addition, as in the NMD data, exenatide did not affect the NO-independent vasodilation, and we propose the protective effect of exenatide on endothelial dysfunction.

In conclusion, we have demonstrated that exenatide administration can induce potent endothelial protection via the opening of K\(_{ATP}\) channels. These findings represent the first human evidence for the effects of exenatide on endothelial pharmacological preconditioning and provide a mechanistic explanation for this phenomenon. Additional studies are necessary to investigate the mechanisms and their potential clinical implications in greater detail.

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**Disclosures**

None.

**References**


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Supplemental Figure 1

- FMD Before IR
  - 250 mmHg for 5 minutes
- IR injury
  - 200 mmHg for 15 minutes
  - Reperfusion for 15 minutes
- FMD After IR
  - 250 mmHg for 5 minutes

- Baseline 1:
  - Radial artery Diameter & velocity
  - BP
  - GMT

- Radial artery Diameter & velocity

- Baseline 2:
  - Radial artery Diameter & velocity

- Radial artery Diameter & velocity
  - BP
  - GMT
Exenatide는 $K_{ATP}$ Channel을 통해 허혈-재관류 손상으로부터 내피세포 기능을 보호한다.

조 현 재 교수
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Summary

배경
허혈-재관류 손상은 내피세포 기능부전을 유발하며, 이 과정에서 $K_{ATP}$ channel이 중요한 역할을 한다고 알려져 있다. 본 연구에서는 exenatide를 투여함으로써 내피세포에 대한 허혈- 재관류 손상을 예방할 수 있는지, 그리고 이러한 보호효과가 $K_{ATP}$ channel을 매개로 이루어지는지 확인하고자 하였다.

방법 및 결과
본 연구는 이중맹검, 위약통제, 교차투여 연구로서, 20명의 지원자들을 대상으로 exenatide 투여군(피하주사 10ug)과 위약군으로 무작위배정 하였다. Exenatide 혹은 위약 투여 30분 후 요골동맥에서 혈관매개확장(flow-mediated dilatation, FMD)을 측정하였다. 상완동맥을 15분간 압박하여 허혈 상태를 유발한 뒤 15분간 재관류 시켰으며, 혈관매개확장은 허혈-재관류 손상의 전후에 각각 측정하였다. Exenatide를 투여한 후, 허혈-재관류 손상 전 12.0±6.23%; 허혈-재관류 손상 후 4.6±3.57% ($P=0.02$). 반면 exenatide 투여 군에서는 허혈-재관류 손상 후에도 혈관매개확장이 저하되지 않아(허혈-재관류 손상 전 15.0±7.14%; 허혈-재관류 손상 후 15.0±5.96%; $P=0.001$) 위약군과 의미 있는 차이를 보였다. 한편, $K_{ATP}$ channel의 차단제인 glibenclamide를 투여한 경우, exenatide를 통한 보호효과가 완전히 소실되는 것을 확인하였다 ($n=7$; 허혈-재관류 손상 전 12.0±2.2%; 허혈-재관류 손상 후 3.2±2.1%; $P=0.001$).

결론
Exenatide 피하주사는 허혈-재관류 손상 인체모델에서 내피세포 기능부전을 예방하며, 이러한 작용은 $K_{ATP}$ channel의 매개로 이루어지는 것을 보여준 연구이다.
협착-재관류 손상은 혈류가 재개통되어 산소 공급이 이루어짐으로써 조직손상이 더욱 악화되는 것을 의미한다. 이전부터 인체의 여러 장기에서 혈착-재관류 손상의 중요성이 알려져 있었으며, 심근경색에서도 혈착 상태의 심근에 재관류치료로 산소공급이 재개될 때 심근 손상이 가중되어 치명적인 합병증이 발생할 수 있다.

현재까지의 연구 결과를 통해, 혈착-재관류 손상에 mitochondria가 중요한 역할을 한다는 사실이 알려져 있다(Figure 1). 혈착 상태에서는 산소공급이 부족하기 때문에 glycolysis를 통해 lactic acid가 증가하고, 그 결과 pH가 저하되어 mitochondria의 ATP 생성이 억제된다. 이에 대한 보상기전으로 Na⁺/H⁺ exchanger (NHE-1)이 activation되어 세포 내 Na⁺가 증가하지만, 이미 ATP 생성이 저하되어 있어서 Na⁺/K⁺ ATPase가 억제되어 있으므로 세포 내 Na⁺를 내보내지 못하게 된다. 증가한 세포 내 Na⁺를 배출시키기 위해 Na⁺/Ca²⁺ exchanger (NCX)가 작용하여, 결국 세포와 mitochondria 내부에 Ca²⁺가 증가한다.

혈착 상태가 지속되는 것은 결국 세포 손상을 야기하지만, 혈착에서 회복되어 재관류가 이루어질 때에 역설적으로 더 큰 손상이 발생할 수 있다. 재관류가 이루어질 때 mitochondria는 다시 ATP를 생성하는데, 이 과정에서 ROS (reactive oxygen species)도 함께 만들어진다. ROS와 Ca²⁺ overload로 인하여 mPTP (mitochondrial

![Figure 1. 혈착-재관류 손상(Ischemia-Reperfusion Injury)의 분자-세포생물학적 기전과 세포 손상을 막기 위한 잠재적 치료 약제.](image-url)
permeability transition pore)가 열리게 되어 mitochondrial swelling이 일어나고, 그 결과 세포 사로 진행하는 것이 바로 재관류 손상의 핵심 기전이라 하겠다.

본 연구는 잘 계획된 이중맹검, 위약통제, 교차투여 연구로서, 당뇨병 치료제로 사용되는 glucagon-like peptide (GLP)-1의 유사체인 exenatide를 건강한 성인에게 투여하고, 혈관매개 확장을 사용하여 허혈-재관류 손상에 대한 보호 효과를 연구하였다. 혈관매개확장은 내피세포 기능을 비침습적으로 정확히 측정할 수 있는 방법으로서, 허혈-재관류 손상 전에 비해 손상 후에 현격하게 저하된다. 하지만 exenatide를 투여한 경우 허혈-재관류 손상 후에도 혈관매개확장이 저하되지 않았고, exenatide와 함께 K<sub>ATP</sub> channel 차단제인 glibenclamide를 투여한 경우에는 이러한 보호효과가 소실되는 결과를 보여주고 있다.

구체적인 분자생물학적 기전을 밝히기 위한 추가연구가 필요하지만, 본 연구를 통해 허혈-재관류 손상을 막는 exenatide의 효과를 확인하였고, 이러한 보호작용이 K<sub>ATP</sub> channel을 매개로 이루어졌음을 알 수 있었다. 이는 허혈-재관류 손상에 mitochondria가 중요한 역할을 담당한다는 사실을 뒷받침하며, 허혈-재관류 손상을 예방할 수 있는 가능성을 제시하여 향후 다양한 실험 및 임상 연구에 활용될 수 있을 것으로 생각된다.

일반적인 허혈-재관류 손상을 막기 위해 adenosine, nicorandil, sildenafil, statin 등의 약제 및 ischemic preconditioning, postconditioning, remote conditioning과 같은 다양한 방법이 시도되어 왔다. 하지만 현재까지 인간을 대상으로 한 연구에서 허혈-재관류 손상을 차단하여 표적 장기를 보호한 결과는 많지 않다.

REFERENCES
Preventive Effects of Exenatide on Endothelial Dysfunction Induced by Ischemia-Reperfusion Injury via $K_{\text{ATP}}$ Channels

Sang Jin Ha, Weon Kim, Jong Shin Woo, Jin Bae Kim, Soo Joong Kim, Woo-Shik Kim, Myeong Kon Kim, Xian Wu Cheng, Kwon Sam Kim

Objective—The purpose of this study was to evaluate whether exenatide administration can prevent impairment in endothelium-dependent vasodilatation induced by ischemia-reperfusion (IR) injury and whether this effect is mediated by $K_{\text{ATP}}$ channel opening.

Methods and Results—In a double-blind, placebo-controlled, crossover design, 20 volunteers were randomly assigned to 2 groups: subcutaneous exenatide (10 μg) or placebo administration. At 30 minutes after the study drug administration, endothelium-dependent flow-mediated dilatation (FMD) of the radial artery was measured before and after IR (15 minutes of ischemia at the level of the brachial artery followed by 15 minutes of reperfusion) injury. Seven days later, both groups were crossed over and received the other treatment (ie, placebo or exenatide) and underwent the same protocol. Pre-IR radial artery diameter, FMD, and baseline radial artery diameter after IR injury were similar between 2 groups ($P$=no significant difference). After placebo administration, IR significantly blunted FMD (before IR: 12.0±6.23%; after IR: 4.6±3.57%; $P=0.02$). Exenatide prevented this impairment (FMD before IR: 15.0±7.14%; after IR: 15.0±5.96%; $P$=no significant difference; $P<0.001$ compared with placebo). In a separate protocol, this protective effect was completely abolished by pretreatment with glibenclamide (glyburide, 5 mg), a blocker of $K_{\text{ATP}}$ channels (n=7; FMD before IR: 12.0±2.2%; after IR: 3.2±2.1%; P<0.001).

Conclusion—The present study demonstrates that subcutaneous exenatide protects IR-induced endothelial dysfunction through opening of $K_{\text{ATP}}$ channels in human IR injury model. (Arterioscler Thromb Vasc Biol. 2012;32:474-480.)

Key Words: endothelium • ischemia • pharmacology • reperfusion injury

Previous studies have emphasized the importance of the vascular endothelium in the pathophysiology of tissue injury induced by ischemia-reperfusion (IR).1 Endothelial cells seem to be more prone to IR injury than cardiac myocytes, and during ischemia the appearance of IR-induced tissue necrosis is temporally preceded by a state of reduced endothelial responsiveness to specific stimuli (endothelial dysfunction).2,3 Many studies have demonstrated that preexposure to brief periods of ischemia (ie, ischemic preconditioning) or specific pharmacological stimuli (pharmacological preconditioning) can adjust myocardial sensitivity to IR injury.4 Furthermore, multiple lines of evidence suggest that stimuli leading to $K_{\text{ATP}}$ channel activation and opening can induce a potent protective effect against IR injury in different cell types. For example, animal models have documented that the administration of specific openers of $K_{\text{ATP}}$ channels leads to a state of reduced susceptibility to IR injury in the endothelium, whereas blockades of these channels with sulfonylureas inhibit endothelial cell ischemic preconditioning.5 Gori et al6 showed that oral sildenafil induces a protection against IR-induced endothelial dysfunction through opening of $K_{\text{ATP}}$ channels and is the first pharmacological preconditioning agent in human.

Exenatide (exendin-4) is a 39–amino acid peptide originally derived from the saliva of the gila monster, a venomous lizard native to the southwestern United States and northern Mexico. Exenatide mimics human glucagon-like peptide (GLP)-1, a gut incretin hormone that is released in response to nutrient intake.7 It exerts insulinotropic and insulinomimetic properties via the G-protein–coupled GLP-1 receptor, which has also been reported to be expressed in the heart.8,9 The GLP-1 receptor has been shown to be cardioprotective in a rat model of myocardial IR injury.10 Timmers et al11 demonstrated compelling evidence that exenatide confers strong cardioprotection and improves left ventricular systolic and diastolic functions in a clinically relevant large animal model of IR injury. However, there was little evidence of the effect of exenatide on the endothelium, especially IR-induced endothelial dysfunction.

In a human in vivo model of IR-induced endothelial dysfunction, we designed the protocols to test (1) whether exenatide administration can prevent impairment in endothelium-dependent vasodilatation induced by IR injury and (2) whether this effect is mediated by $K_{\text{ATP}}$ channel opening.
Lium-dependent vasodilatation induced by IR injury and (2) whether this effect is mediated by $K_{ATP}$ channel opening.

**Materials and Methods**

The ethics committee of the Kyung Hee University approved this study, and written informed consent was obtained in all cases. Studies were conducted in a quiet, temperature- and humidity-controlled environment.

**Protocol 1: The Effect of Exenatide on IR Injury in the Endothelium**

Twenty healthy nonsmoking volunteers (25–40 years old) were enrolled in this double-blind, randomized, placebo-controlled crossover trial. All subjects except for 1 who had been previously shown to be able to completely inhibit forearm $K_{ATP}$ channels.9 With the glibenclamide administration, a 10% dextrose infusion was started and was titrated to maintain blood sugar levels between 80 and 120 mg/dL throughout the study period. Initially, radial artery endothelium-dependent flow-mediated dilatation (FMD) and endothelium-independent nitroglycerin-mediated dilatation (NMD) were measured as described in detail below. Subsequently, 1.5 hours later, IR injury was performed by a maneuver which placed a pneumatic cuff at the level of the brachial artery and inflated to 200 mm Hg for 15 minutes to induce radial artery ischemia. At the end of ischemia, 15 minutes of reperfusion was performed to induce reperfusion injury. After IR injury, radial artery FMD was measured again (Supplemental Figure I, available online at http://atvb.ahajournals.org). All volunteers had a wash-out period of 7 days. Seven days later, the subjects returned to crossover study medication (ie, exenatide or placebo), and the protocol described above was repeated.

Blood samples (for insulin analysis) and supine blood pressure were taken to record at 3 points (before examination, after baseline FMD and NMD, and after the end of examination) in protocol 1. Plasma insulin levels were assessed by ELISA (BenderMed Systems).

**Protocol 2: Effect of Glibenclamide**

In a separate protocol, 7 healthy volunteers were administered 5 mg of glibenclamide (glyburide, Euglucon, Roche Pharma) 1 hour before administration of 10 µg of exenatide. This dosage has previously been shown to be able to completely inhibit forearm $K_{ATP}$ channels.9 With the glibenclamide administration, a 10% dextrose infusion was started and was titrated to maintain blood sugar levels between 80 and 120 mg/dL throughout the study period. One and a half hours after exenatide administration, the subjects underwent FMD measurement before and after IR, as described above. Because of safety concerns related to the potent hypoglycemic effect of glibenclamide requiring continuous adjustment of a dextrose infusion, this protocol was not double-blind. All subjects except for 1 also participated in protocol 1.

**Measurement of Radial Artery Diameter and FMD**

Subjects were asked to rest for 10 minutes in the supine position before each study protocol. The FMD and NMD measurements were performed as follows by 2 independent operators. Radial artery images were performed using commercially available system (Vivid 7, GE Vingmed, Horten, Norway) equipped with a 14-MHz linear array transducer. The ECG-gated, end-diastolic, longitudinal, B-mode images were digitally stored on the hard disk of the instrument for online and offline analysis. For FMD measurement, the baseline radial artery diameter was averaged from 6 separate images taken at 5-second intervals. Subsequently, a pneumatic cuff placed at the level of the wrist (ie, distal to the site of radial artery measurement) was inflated to 250 mm Hg for 5 minutes. After wrist-cuff deflation, radial artery diameter was reexamined and averaged from 6 separate images taken at 5-second intervals. FMD was calculated as the percentage maximum increase in arterial diameter. After a 10-minute rest period to allow restoration of baseline conditions, NMD was assessed by obtaining 2-dimensional images before and 3 minutes after administration of 50 µg of GTN for determining endothelial independent vasodilatation.3 Radial artery diameter was calculated from the trailing edge of the intima-blood interface to the leading edge semiautomatically using a modified version of ImageJ software (National Institutes of Health), as well as custom-designed software. Baseline and post-IR (reactive hyperemia) radial blood flows were measured using pulsed-wave Doppler as an average velocity-time integral for the first 5 cardiac cycles after cuff deflation and were multiplied by heart rate and vessel cross-sectional area.

A flow chart illustrating the measurement protocol and time points is shown in Supplemental Figure I.

**Statistical Analysis**

All of the data are presented as the mean±standard deviation. Baseline values were compared by use of a paired $t$ test (between visits, protocol 1) or an unpaired $t$ test (between protocols). The effect of IR on radial artery diameters, reactive hyperemia, and FMD within each study visit (protocols 1 and 2) was tested by use of a paired $t$ test. One-way ANOVA (comparison of 3 or more groups for FMD) followed by the Tukey post hoc test was used for statistical analysis. Between-group differences (exenatide versus placebo and pre-IR versus post-IR) of artery diameter and blood flow in protocol 1 were analyzed by use of a 2-way ANOVA for repeated measures. A value of $P<0.05$ was set as the threshold for significance. The analyses were performed using software (SPSS version 17.0, SPSS, Chicago, IL).

**Results**

The radial artery diameter and blood flow measurements are presented in Tables 1 and 2, and FMD data are reported in Figures 1 and 2. To confirm interobserver and intraobserver correlations for reproducibility, we performed measurements of the same data by 2 other people and repeated the measurements 3 months later. There was good correlation between the interobserver and intraobserver coefficients ($R=0.9$).

**Protocol 1**

**Effects of Exenatide Administration on the Baseline Parameters**

Exenatide administration had no effect on arterial blood pressure (placebo 113±5 mm Hg versus exenatide 116±7 mm Hg; placebo 77±7 mm Hg versus exenatide 75±16 mm Hg; $P=$no significant difference [NS]). Also, exenatide had no effect on radial artery diameter, FMD, or NMD before IR (Table 1 and Figure 1, $P=$NS). However, exenatide had an effect on blood flow as expressed in reactive hyperemia, shown in Table 2 (50.7±57.2% versus 210±120%, $P<0.001$).

**Effects of IR After Placebo Administration**

In the placebo group, radial artery diameter and blood flow values were not different from baseline values before and after IR ($P=$NS). But post-IR FMD was significantly reduced when it was compared with the pre-IR level (before IR: 12.0±6.23%; after IR: 4.6±3.57%, $P=0.02$, Table 1 and Figure 1). Also, decreased reactive hyperemia was noted (before IR: 50.7±57.2%; after IR: 23.6±32.3%, $P<0.05$, Table 2) but no significant change in NMD was observed.
Effects of IR After Exenatide Administration

In the exenatide group, radial artery was dilated after IR injury (P=NS compared with exenatide before IR and with placebo after IR). IR significantly affected the peak reactive hyperemia despite of exenatide administration (before IR: 210±120%; after IR: 55.3±32.5%, P<0.001 compared with before IR, Table 2), but there was no statistical difference in NMD between the placebo and the exenatide groups (P=NS, Table 2). Although IR injury significantly reduced blood flow, exenatide administration completely restored the FMD reduction that was observed in placebo group after IR injury (Figure 1; P=NS compared with exenatide before IR; P<0.05 compared with placebo after IR; 2-way ANOVA) without effect on NMD. During the protocol, insulin level at the point of first FMD measurement and end of examination increased significantly and gradually compared with baseline insulin level before examination (3.6±3.5 versus 22.0±4.9 versus 34.3±5.8 μU/mL, P<0.05). However, there was no statistical difference in the blood sugar levels among baseline, 1st FMD measurement, and end of examination (90±5.7 versus 85±3.5 versus 82±2.3 mg/dL, P=NS). Exenatide had

Table 1. Results of FMD and NMD in Protocols 1 and 2

<table>
<thead>
<tr>
<th>Artery Diameter Pre-IR</th>
<th>Artery Diameter Post-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage</td>
</tr>
<tr>
<td>FMD</td>
<td></td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>12.0±6.23</td>
</tr>
<tr>
<td>Exenatide</td>
<td>15.0±7.14</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
</tr>
<tr>
<td>Glyben + exenatide</td>
<td>12.02±2.16</td>
</tr>
<tr>
<td>NMD</td>
<td></td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>10.4±3.24</td>
</tr>
<tr>
<td>Exenatide</td>
<td>10.8±0.42</td>
</tr>
</tbody>
</table>

Values are mean±standard deviation. FMD indicates flow-mediated dilatation; NMD, nitroglycerin-mediated dilatation; IR, ischemia-reperfusion.
*P<0.05 vs corresponding controls (before IR).
†P<0.05 vs corresponding controls (placebo in protocol 1).

Table 2. Results of Blood Flow of the Radial Artery in Protocols 1 and 2

<table>
<thead>
<tr>
<th>Blood Flow</th>
<th>Baseline Blood Flow (mL/min)</th>
<th>After Wrist Cuff Deflation (mL/min)</th>
<th>Reactive Hyperemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>177.3±98.9</td>
<td>252.4±139.1</td>
<td>50.7±57.2</td>
</tr>
<tr>
<td>Exenatide</td>
<td>186.2±103.9</td>
<td>524.4±247.9*</td>
<td>210±120*</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyben + exenatide</td>
<td>184.2±138.6</td>
<td>237.4±166.9</td>
<td>50.9±28.1</td>
</tr>
<tr>
<td>Post-IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>172.9±89.7</td>
<td>206.8±109.7†</td>
<td>23.6±32.3†</td>
</tr>
<tr>
<td>Exenatide</td>
<td>163.1±12.4</td>
<td>247.5±141.7††</td>
<td>55.3±32.5†</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyben + exenatide</td>
<td>165.6±148.6</td>
<td>227.5±157.5†</td>
<td>23.4±27.4†</td>
</tr>
</tbody>
</table>

Value are mean±standard deviation. IR indicates ischemia-reperfusion.
*P< 0.05 vs corresponding controls (placebo in protocol 1).
†P<0.01 vs corresponding controls (baseline blood flow).
‡P<0.05 vs corresponding controls (pre-IR).

Figure 1. Box plots of the flow-mediated dilatation (FMD) responses before and after ischemia-reperfusion (IR) (Protocol 1). Left: In the placebo group, FMD was significantly blunted after IR. Right: Exenatide completely prevented this impairment in endothelium-dependent vasodilation induced by IR (Tukey-type multiple comparison test). Boxes show interquartile ranges; the lower and upper boundaries of the boxes indicate the 25th and 75th percentile levels, respectively; and the horizontal lines within the boxes indicate the median levels.
Figure 2. Box plots of the flow-mediated dilatation (FMD) responses before and after ischemia-reperfusion (IR) following administration of glibenclamide and exenatide (protocol 2). FMD was significantly blunted after IR, demonstrating that glibenclamide inhibited exenatide-induced endothelial protection against IR. Boxes show interquartile ranges; the lower and upper boundaries of the boxes indicate the 25th and 75th percentile levels, respectively; and the horizontal lines within the boxes indicate the median levels.

no effect on radial artery diameter, FMD, or NMD before IR (Table 1 and Figure 1, P=NS).

Protocol 2

Effect of Glibenclamide
Radial artery diameter and blood flow data are reported in Tables 1 and 2. Although reactive hyperemia significantly different (exenatide only: 210±120%; glibenclamide+exenatide: 51±28%, P<0.001, Table 2), pre-IR baseline radial artery diameter and FMD were not different after glibenclamide plus exenatide compared with exenatide alone. After IR, baseline radial artery diameter returned to the values observed before IR. However, despite the greater blood flow stimulus caused by the exenatide, FMD was blunted to much lower values than those observed in the placebo group of protocol 1, demonstrating a potent inhibitory effect of $K_{\text{ATP}}$ channel blockade on exenatide-induced endothelial protection (exenatide only: 12.0±2.2%; glibenclamide+exenatide: 3.2±2.1%, P<0.001 compared with before IR, Figure 2). In addition, this blunting effect of glibenclamide overcame the increased blood flow effect of exenatide (Table 2). These results demonstrated that exenatide is $K_{\text{ATP}}$ channel dependent (glibenclamide sensitive). During protocol 2, no hypoglycemic events developed, and blood sugar level was maintained (before protocol 1: 97.0±3.5 mg/dL; end of protocol: 82±2.3 mg/dL).

Discussion
This study showed that exposure to IR impaired FMD at the level of the forearm conductance vessels and that impaired FMD caused by IR can be prevented by pretreatment with exenatide, which mimics ischemic preconditioning at the endothelium level; this effect is blunted by the $K_{\text{ATP}}$ channel blocker glibenclamide. To the best of our knowledge, this is the first report demonstrating that exenatide induces potent protection against IR-induced endothelial dysfunction through the opening of $K_{\text{ATP}}$ channels.

Conduit arteries (eg, the brachial, radial, femoral, or popliteal) dilate in response to an increase in blood flow.12-14 This physiological response is dependent on the presence of an intact endothelium,15,16 and the measurement of FMD in vivo has been widely adopted as an assessment of endothelial function.17 Peripheral artery disease, a manifestation of atherosclerosis, is a very common comorbidity seen in the setting of coronary artery disease because the 2 conditions share a similar pathogenesis, including endothelial dysfunction.18,19 It is well known that the examination of FMD in peripheral artery is the most widely used noninvasive ultrasound method to assess endothelial function.20,21 Previously, Kuvin et al22 reported that peripheral vascular endothelial function testing as noninvasive indicator of coronary artery disease. Furthermore, it has recently been reported that impaired FMD is the only significant factor associated with acute ST-elevation myocardial infarction.23 It seems to be that the standard FMD model used here could be applied to not only evaluate peripheral but also coronary artery endothelial dysfunction.

The results of cardiac IR injury are not restricted to myocytes but also affect the coronary endothelium, where they are characterized by decreased nitric oxide (NO)–dependent relaxations. Given the key role of the endothelium and NO in the control of vascular tone, as well as platelet and leukocyte functions, protection of coronary endothelial cells is an important therapeutic target in IR injury.24,25 The pathophysiological significance of reperfusion-induced coronary endothelial injury of large coronary arteries may be related, at least in part, to the properties of endothelium-derived NO at this level. Indeed, constitutive NO production continuously opposes vasoconstrictor influences in large coronary arteries.26 Moreover, NO has a inhibitory effect on platelet aggregation and leukocyte activation and adhesion.27,28 Thus, endothelial dysfunction may lead to vasoconstriction and increased platelet aggregation, resulting in subsequent increased risks of vasospasm and thrombosis, and NO has the protective effects of preconditioning against reperfusion development of atherosclerosis.29,30

In a previous report, Kharbanda et al31 demonstrated that a clinically relevant period of IR causes profound and sustained endothelial dysfunction assessed by FMD at the level of the forearm conductance vessels while leaving endothelium-independent vasodilation unchanged. In their report, exposure to brief episodes of ischemia (ie, ischemic preconditioning) attenuates this specific impairment in endothelium-dependent relaxation. Animal studies have demonstrated that administration of pharmacological stimuli, such as adenosine, bradykinin, nitric oxide donors, and opioids, can induce a protective phenomenon analogous to ischemic preconditioning.29 Importantly, a key stage in the complicated molecular pathways activated by these mediators, as well as by ischemic preconditioning, seems to be the opening of $K_{\text{ATP}}$ channels. As a result, mitochondrial $K_{\text{ATP}}$ opening evokes a response involving several different protective mechanisms, including matrix swelling, reactive oxygen species modulation, and
effects on mitochondrial Ca$^{2+}$ homeostasis.$^{5,30,31}$ Interestingly, multiple lines of evidence have emphasized the role of endogenous (endothelial) and exogenous nitric oxide in the physiologies of both ischemic and pharmacological preconditionings$^{32}$ directly and via opening of K$_{ATP}$ channels.$^{30,33}$ This pathway might have clinical relevance, because (1) administration of a nitric oxide donor can reduce myocardial ischemia during angiplasty,$^{34}$ and (2) administration of K$_{ATP}$ channel blocker sulfonylureas, such as glibenclamide, prevents the development of cardiac ischemic preconditioning.$^{35}$ Downey et al$^{36}$ have developed the hypothesis that stimulation of a variety of G protein–coupled receptors results in the activation of protein kinase C. This, in turn, leads to the translocation of protein kinase C from the cytoplasm to the sarcolemma, where it phosphorylates a substrate protein (possibly the ATP-sensitive K1 [K$_{ATP}$] channel), which confers resistance to ischemia.

Exenatide/exendin-4 and GLP-1 are known to have a number of cardiovascular effects.$^{11,37,38}$ In isolated perfused rat hearts, exendin-4 reduced infarct size when administered at reperfusion.$^{37}$ Experiments in both isolated and in vivo rat hearts suggest that GLP-1 significantly attenuates reperfusion injury and reduces infarct size by approximately 50% when coadministered with a dipeptidyl peptidase-IV inhibitor.$^{10}$ In recent research, Timmers et al$^{11}$ presented data in which the incretin mimetic exenatide significantly reduces myocardial infarction and improves left ventricular contraction and relaxation when it is given at reperfusion. Emerging lines of evidence show an additional benefit of GLP-1 on the endothelium. Indeed, except for cardiomyocytes, GLP-1R expression has been detected on endothelial and vascular smooth muscle cells, as well as on macrophages and monocytes.$^{39}$ Ban et al$^{38}$ proposed a novel 2-pathway schema for cardiovascular actions of GLP-1: in 1 pathway, binding to the GLP-1 receptor mediates effects on cardiac inotropic action, glucose uptake, ischemic preconditioning and mild vasodilatation; the other pathway depends on the rapid breakdown of GLP-1 to GLP-1(9–36) and the consequent receptor-independent effects on the posts ischemic recovery of cardiac function and vasodilatation. GLP-1(9–36) also appeared to improve the survival of human aortic endothelial cells after IR.$^{40}$ These actions were also exerted through the nitric oxide synthase pathway.$^{38}$ Nevertheless, some investigators observed a vasodilatory effect of GLP-1 independently of NO, indicating clearly a direct action on vascular smooth muscle cells via its GLP-1R.$^{41}$ In particular, in type 2 diabetes mellitus patients with coronary artery disease, rGLP-1 infusions (at a dose of 2 pmol/kg per minute) significantly increased FMD in the brachial arterial compared with placebo, but in healthy subjects, GLP-1 infusion did not affect the FMD.$^{42}$ Recent studies demonstrated that exenatide had the blood pressure lowering effect and could be applied to diabetes patients.$^{43}$ Furthermore, GLP-1 infusion enhanced acetylcholine-mediated vasodilation in nondiabetic, normotensive nonsmokers, an effect that was abolished after administration of glyburide (but not glimepiride). These data indicate also a potential modulatory role of sulfonylurea receptor subunit on GLP-1Rs in the endothelial cells and a selectivity of K$_{ATP}$ channel inhibition among different sulfonylurea agents.$^{44}$

In our study, exenatide (or the combination of exenatide and glibenclamide) had no effect on baseline radial artery diameter, NMD, and FMD but increased the blood flow, expressed as reactive hyperemia. In the point of the view that the reactive hyperemia is the stimulus for FMD, it is likely that there was discrepancy in the FMD and reactive hyperemia data. Further study will be required to investigate for this issue. Additionally, it has been reported that GLP-1 infusion had no effect on FMD and nitroglycerin responses in the healthy subjects, whereas it improved endothelial dysfunction in type 2 diabetes mellitus with coronary heart disease.$^{42}$ As in the our protocol dealing with healthy subjects, there was no effect of GLP-1 pretreatment on FMD and NMD-mediated response. On the contrary, the endothelial dysfunction induced by IR injury was not observed in the exenatide pretreatment group. These findings suggested that the pretreatment with exenatide prevented endothelial dysfunction induced by IR injury.

The effect of GLP-1 on blood flow can be explained by a previous animal experiment showing that GLP-1 induces potent relaxation of rat conduit arteries via a specific receptor independently of NO and the endothelium.$^{41}$ Previous studies have shown that the IR injury used here specifically impairs endothelium-dependent response while leaving endothelium-independent reactivity unaltered.$^{3}$ Therefore, the fact that reactive hyperemia, a predominantly NO-independent process,$^{45}$ is unimpaired in the present study should be expected. However, radial artery FMD after IR was significantly reduced in the placebo group, whereas the administration of exenatide remarkably limited this effect. These data demonstrate that although IR is able to induce a significant impairment in endothelium-dependent vasodilatation, pretreatment with exenatide can protect the endothelium from IR injury via a specific molecular pathway. As in our data from protocol 2, the endothelial protective effect of exenatide was almost completely prevented when a K$_{ATP}$ channel blocker was administered before the exenatide. Although hyperglycemia induces oxidative and inflammatory stress and suppresses endothelial nitric oxide synthase, we maintained the normoglycemic level throughout the experiment. This demonstrated that exenatide is K$_{ATP}$ channel-dependent (glibenclamide-sensitive) and it is likely that the protective effect of exenatide is mediated by K$_{ATP}$ channel opening. Overall, the results suggest that exenatide exerts the ischemic preconditioning effect through the NO-dependent pathway, of which K$_{ATP}$ channels are a key effector molecule. It should be noted that 1 likely explanation for the higher FMD of IR with exenatide is a difference in the flow stimulus, as this was statically higher and is the stimulus for dilation. This suggests that an NO-independent effect may be responsible for exenatide-mediated higher FMD after IR.

With the exenatide and glibenclamide administration, a 10% dextrose infusion was maintained and was titrated to maintain blood sugar levels between 80 and 120 mg/dL throughout the study period. As we are well aware, the action of the GLP-1 analog exenatide increases insulin secretion from $\beta$-cells via glucose-dependent stimulation of insulin
secretion mechanism. The half-life of exenatide is 2.4 hours, and it raises insulin levels quickly (within ~10 minutes of administration), with the insulin levels subsiding substantially over the next hour or 2. In our data, insulin levels gradually increased at the end of measurement protocol, 55 minutes after exenatide injection.

Our data were collected from forearm blood circulation in healthy volunteers, so direct comparison with coronary circulation has some limitations. The study model does not show the specific molecular mechanisms involved in the protective effect. The exact cellular and subcellular sites on which exenatide and glibenclamide act, as well as the exact mediators involved, will have to be investigated with in vitro studies. However, because previous studies have demonstrated that the IR protocol used here does not affect endothelium-independent vascular responses and because of the evidence that endothelial cells, like smooth muscle cells, possess the apparatus necessary to develop preconditioning, we propose a direct involvement of endothelial cells in our model. In addition, as in the NMD data, exenatide did not affect the NO-independent vasodilation, and we propose the protective effect of exenatide on endothelial dysfunction.

In conclusion, we have demonstrated that exenatide administration can induce potent endothelial protection via the opening of KATP channels. These findings represent the first human evidence for the effects of exenatide on endothelial pharmacological preconditioning and provide a mechanistic explanation for this phenomenon. Additional studies are necessary to investigate the mechanisms and their potential clinical implications in greater detail.

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Disclosures
None.

References


