Exercise improves the dilatation function of mesenteric arteries in postmyocardial infarction rats via a PI3K/Akt/eNOS pathway-mediated mechanism

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Submitted 14 July 2010; accepted in final form 29 September 2010

Wang Y, Wang S, Wier WG, Zhang Q, Jiang H, Li Q, Chen S, Tian Z, Li Y, Yu X, Zhao M, Liu J, Yang J, Zhang J, Zang W. Exercise improves the dilatation function of mesenteric arteries in post-myocardial infarction rats via a PI3K/Akt/eNOS pathway-mediated mechanism. Am J Physiol Heart Circ Physiol 299: H2097–H2106, 2010. First published October 8, 2010; doi:10.1152/ajpheart.00701.2010.—Myocardial infarction (MI) has been shown to induce endothelial dysfunction in peripheral resistance arteries and thus increase peripheral resistance. This study was designed to investigate the underlying mechanisms of post-MI-related dysfunctional dilatation of peripheral resistance arteries and, furthermore, to examine whether exercise may restore dysfunctional dilatation of peripheral resistance arteries. Adult male Sprague-Dawley rats were divided into three groups: sham-operated, MI, and MI + exercise. Ultrastructure and relaxation function of the mesenteric arteries, as well as phosphatidylinositol-3 kinase (PI3K), Akt kinases (Akt), endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) production, and phosphorylation of PI3K, Akt, and eNOS by ACh were determined. Post-MI rats exhibited pronounced ultrastructural changes in mesenteric artery endothelial cells and endothelial dysfunction. In addition, the activities of PI3K, Akt, and eNOS, and their phosphorylation by ACh were significantly attenuated in mesenteric arteries (P < 0.05–0.01). After 8 wk of exercise, not only did endothelial cells appeared more normal in structure, but also ameliorated post-MI-associated mesenteric arterial dysfunction, which were accompanied by elevated activities of PI3K, Akt, and eNOS, and their phosphorylation by ACh (P < 0.05–0.01). Importantly, inhibition of either PI3K or eNOS attenuated exercise-induced restoration of the dilatation function and blocked PI3K, Akt, and eNOS phosphorylation by ACh in the mesenteric arteries. These data demonstrate that MI induces dysfunctional dilatation of peripheral resistance arteries by degradation of endothelial structural integrity and attenuating PI3K-Akt-eNOS signaling. Exercise may restore dilatation function of peripheral resistance arteries by protecting endothelial structural integrity and increasing PI3K-Akt-eNOS signaling cascades.

ISCHEMIC HEART DISEASE (IHD) is a leading cause of death worldwide, especially in developed countries (40, 47). Most patients with IHD eventually progress to irreversible cardiac dysfunction and heart failure, and this has presented a particularly challenging issue for the prevention and treatment of IHD (23, 26, 31). Considerable evidence from animal experiments and clinical observations has demonstrated endothelial dysfunction in peripheral resistance arteries following myocardial infarction (MI) (6, 42). This most likely contributes to increased peripheral arterial vasoconstriction and thus increased peripheral resistance (49, 50). This pathophysiological change has been implicated in the development of post-MI chronic cardiac dysfunction and resultant heart failure by increasing the afterload and aggravating heart failure (42). Therefore, elucidation of the mechanisms underlying post-MI-related dysfunctional dilatation in peripheral vessels may provide some valuable clues for the prevention and treatment of IHD.

Numerous studies have demonstrated that endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) production from endothelial cells through NADPH-dependent oxidation of L-arginine plays a pivotal role in vasorelaxation (28, 30, 55), and that phosphatidylinositol-3 kinase (PI3K) and Akt are important upstream regulators of eNOS activation (11, 29, 34). Under normal circulation conditions, shear stress exerted by intraluminal flow represents a permanent stimulus for eNOS-derived NO production by endothelial cells (3, 4, 10, 17, 52). At the cellular level and in isolated blood vessels, it is well established that shear stress increases NO production by activating the PI3K-Akt signaling pathway that leads to eNOS phosphorylation (7, 12, 42). In contrast, the loss of the Akt1 subtype in the vessel wall is associated with reduced eNOS phosphorylation (8), and MI or other diseases that produce oxidative stress have been shown to contribute to endothelial dysfunction and degraded ultrastructure of mesenteric endothelial cells (16, 20, 38). Inducing dysfunction of the eNOS-NO system by inhibition upstream signaling, impairing eNOS directly, or decreasing NO bioavailability has also been shown to cause dysfunctional vasorelaxation. Despite the well-known importance of the eNOS-NO system in vascular physiology and pathophysiology, it remains unclear if the post-MI vascular dysfunction is secondary to a perturbation of the eNOS-NO system.

Despite a high mortality in patients with MI, increasing survival has been accomplished with improved treatments, such as pharmacological intervention and exercise training (33, 35, 51). Both experimental studies and clinical observations have indicated that exercise, especially low-intensity aerobic exercise, benefits vascular function (22, 23, 25, 43, 44, 55). The mechanisms underlying this improvement are thought to involve a variety of cellular and molecular alterations, including an upregulation of eNOS and its phosphorylation, an
increase in the expression and/or activity of antioxidant enzymes, and a decrease in prooxidant enzyme systems (5, 22). The most satisfactory explanation for exercise-induced improvement of vascular function is that exercise increases shear stress by increasing blood flow and thus stimulates the vascular PI3K and Akt pathway (15). Although the stress-response machinery is not well defined, the activation of PI3K-Akt signaling may further activate intracellular calcium-independent eNOS, which is involved in an exercise-mediated shear stress-activated signal transduction cascade (15). This hypothesis is further supported by our laboratory’s study showing that Akt contributes to vascular eNOS Ser1177 phosphorylation in treadmill-running animals (55). However, it is has yet to be determined if exercise may also activate PI3K-Akt-eNOS signaling cascades and thus restore dysfunction relaxation of peripheral resistance vessels following MI.

Therefore, in the present study, we sought to determine whether: 1) MI causes dysfunctional vasorelaxation and degradation of endothelial ultrastructure in peripheral resistance arteries; 2) MI attenuates PI3K-Akt-eNOS-NO signaling cascades; and 3) exercise can restore endothelial structural degradation, activate the PI3K-Akt-eNOS-NO signaling cascade, and thus ameliorate dysfunctional vasorelaxation.

MATERIALS AND METHODS

Drugs and chemicals. LY-294002, N\textsuperscript{2-}nitro-L-arginine methyl ester (L-NAME), ACh, triphenyltetrazolium chloride (TTK), and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO). Antibodies against PI3K p85 subunit, phosphorylated PI3K p85 subunit (p-PI3K), Akt, phosphorylated-Akt (Ser473) (p-Akt), eNOS, and phosphorylated-eNOS (Ser1177) (p-eNOS) were obtained from Cell Signaling Technology (Beverly, MA). NO assay kit was from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Bicinchoninic acid protein assay kit was from Pierce (Rockford, IL, USA).

Animal MI model. Adult male Sprague-Dawley rats (6 wk old), weighing 220 ± 18 g (purchased from the Experimental Animal Center of Xi’an Jiaotong University, China), were used in this study. All animals were housed individually in a temperature-controlled animal room (22–24°C) under a 12-h light (730 –1930)/12-h dark (1930 –730) circadian cycle, with free access to chow and water. All experimental procedures and protocols conformed to the recommended guidelines on the care and use of laboratory animals issued by the Chinese Council on Animal Research. The study was approved by the ethical committee of Xi’an Jiaotong University. The protocol of MI was created by ligation of the left anterior descending coronary artery (LAD) (48). Briefly, rats were anesthetized with 2% isoflurane and then quickly cut into 3- to 5-mm sections with a razor blade. After TTC staining, the area of infarction exhibited a white color, whereas noninfarcted areas were red.

Preparation of mesenteric arterial rings and isometric tension measurement. Mesenteric arterial rings were prepared after hemodynamic measurements. Small superior mesenteric resistance arteries were gently isolated and immersed immediately in cold oxygenated Krebs solution (119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 25 mM NaHCO\textsubscript{3}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, and 11 mM d-glucose). Arteries were carefully cleaned of fat and connective tissue and cut into 1- to 2-mm-length rings. Endothelium was removed by gentle scraping with a stainless steel wire inserted into the artery. Removal of the endothelium was confirmed by a lack of relaxation response to 1 μM ACh. Isometric tension was measured as previously described (45). Briefly, arterial rings (internal diameter ~150 μm) were mounted in a Multi Myograph System (Danish Myotechnology A/S, Aarhus, Denmark), and changes in isometric tension were continually recorded. Two tungsten wires were passed through the lumen of the ring. One of the wires was fixed to a micrometer for length adjustments, and the other was connected to a force transducer for isometric force measurements. Each ring was bathed in an organ chamber containing Krebs solution, maintained at 37°C, and continuously bubbled with 95% plus 5% CO\textsubscript{2} (pH 7.4). After mounting with a previously determined optimal resting tension of 4 mN for 60 min, each ring was first contracted by 10 μM phenylephrine (PE) and then challenged with 1 μM ACh to confirm the vessel’s contractility and the integrity of its endothelium. The rings were then washed to restore tension to baseline and allowed to stabilize for 60 min. Thereafter, the rings were preconstricted with PE (0.1–2 μmol/l) to comparable constriction levels in each group, and relaxant responses to cumulative doses of ACh and to sodium nitroprusside (SNP) were assessed. Cumulative concentration-response curves to ACh, SNP, PE, or CaCl\textsubscript{2} were obtained both in intact and in endothelium-denuded mesenteric artery rings. The PI3K-specific inhibitor, LY-294002, was dissolved in DMSO and diluted in saline containing 0.1% DMSO. In another experiment, mesenteric artery rings were incubated for 15 min with inhibitors LY-294002 (20 μM) or L-NAME (0.2 mM) before being stimulated with ACh to study the effects of the blockade of PI3K or eNOS, respectively, on ACh-induced vascular relaxation.

Hematoxylin and eosin staining, immunohistochemistry, and electron microscopy. Rats were anesthetized with pentobarbital sodium (30 mg/kg iv). Rat superior mesenteric arteries were carefully isolated, and part of the mesenteric artery was fixed with 4% paraformaldehyde for <48 h. Fixed mesenteric artery segments were dehy-
did not change (Table 1), ligation for 8 wk induced a MI that occupied an average of 41% of cardiac tissue. SBP, DBP, LVSP, and ±dP/dt\(_{max}\) were markedly attenuated, and LVEDP was increased compared with Sham (P < 0.05–0.01), indicating that 8-wk ligation caused evident cardiac dysfunction.

To determine the existence of dysfunctional dilation of peripheral resistance arteries in animals post-MI, we isolated mesenteric arteries from Sham and post-MI animals and measured ACh-induced vasorelaxation. As shown in Fig. 1, increasing concentrations of ACh progressively relaxed vessels to a greater and greater extent, reaching a maximal relaxation of 89.5 ± 4.4% at 10\(^{-5}\) M ACh. Relaxation was an endothelium-dependent process, shown by the fact that mechanical denudation of endothelium almost abolished ACh-induced vasorelaxation, resulting in maximal vasorelaxation of 16.8 ± 4.6% (Fig. 1A). ACh-induced vasorelaxation was significantly attenuated in mesenteric arteries from post-MI animals compared with Sham (66.1 ± 5.4 vs. 89.5 ± 4.4%; P < 0.05), suggesting the existence of post-MI-associated dysfunctional dilation of peripheral resistance arteries (Fig. 1A). The endothelium dependence of the process suggests that post-MI-associated dysfunctional dilation may originate from the impaired endothelium. To test this hypothesis, we measured vasorelaxation in the presence of SNP, a NO donor that induces endothelium-independent vasorelaxation (Fig. 1E). As expected, SNP relaxed post-MI and Sham mesenteric arteries to a comparable extent, indicating that dysfunctional dilation of mesenteric arteries was primarily due to the impairment of endothelial rather than to impairment of the smooth muscle layer. In addition, removal of the endothelium did not significantly modify SNP-induced vasorelaxation (data not shown).

Mesenteric artery endothelial cells show degraded structure in post-MI animals. Previous evidence has indicated that structural changes in cells often cause functional changes (38). To test whether mesenteric artery endothelial cells in post-MI animals also showed structural degradation, we assessed the ultrastructure of mesenteric artery endothelial cells with transmission electron microscopy (JEM-2000EX).

Table 1. Effects of myocardial infarction and exercise training on animal characteristics and hemodynamic parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>MI</th>
<th>MI+Ex</th>
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<tbody>
<tr>
<td>Infarct size, %</td>
<td>41 ± 3</td>
<td>39 ± 2</td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>413 ± 8</td>
<td>402 ± 11</td>
<td>422 ± 7§</td>
</tr>
<tr>
<td>Ht/Wt, g</td>
<td>1.3 ± 0.06</td>
<td>1.4 ± 0.06</td>
<td>1.5 ± 0.10</td>
</tr>
<tr>
<td>Ht/Wt, g/BW, kg</td>
<td>3.2 ± 0.26</td>
<td>3.6 ± 0.41*</td>
<td>3.5 ± 0.24</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>407 ± 13</td>
<td>413 ± 17</td>
<td>396 ± 11‡</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>122 ± 1.7</td>
<td>105 ± 2.5*</td>
<td>114 ± 3.2‡</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>88 ± 1.0</td>
<td>78 ± 1.9*</td>
<td>82 ± 2.8</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>126 ± 3.7</td>
<td>106 ± 1.6*</td>
<td>118 ± 3.6‡</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>5 ± 1.1</td>
<td>19 ± 2.3†</td>
<td>14 ± 1.8‡</td>
</tr>
<tr>
<td>LV +dP/dt(_{max}), mmHg/s</td>
<td>8,772 ± 301</td>
<td>5,568 ± 273†</td>
<td>6,239 ± 451§</td>
</tr>
<tr>
<td>LV -dP/dt(_{max}), mmHg/s</td>
<td>-7,346 ± 481</td>
<td>-4,478 ± 498</td>
<td>-5,276 ± 637</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 for both groups. Animal characteristics and hemodynamic parameters are given in the sham operation (Sham), myocardial infarction (MI), and myocardial infarction + exercise training (MI+Ex) groups. BW, body weight; Ht/Wt, heart weight; Ht/BW, heart weight-to-body weight ratio; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; LV +dP/dt\(_{max}\), positive and negative maximal values of the instantaneous first derivative of left ventricular pressure. *P < 0.05, †P < 0.01 vs. Sham. ‡P < 0.05, §P < 0.01 vs. MI.

RESULTS

Cardiac infarction induces dysfunctional dilation in peripheral resistance arteries. To investigate the mechanisms underlying dysfunctional dilation of peripheral resistance arteries in myocardial ischemia, we ligated the LAD ~3 mm below the left atrium for 8 wk to mimic postmyocardial ischemia-cardiac dysfunction. Results showed that, although whole heart weight...
mission electron microscopy. As shown in Fig. 2, compared with Sham, some endothelial cells were edematous, resulting in a rough cell surface; in some areas, smooth muscle layers were directly exposed to the vessel lumen, because of endothelial cell desquamation. Within endothelial cells of post-MI animals, there were numerous vacuoles and lysosomes, and nuclei displayed digitations and chromatin abnormalities. In addition, the diffusible distribution of collagen fibers was observed in the extracellular matrix of endothelium of post-MI rats (Fig. 2H). These results indicate structural degradation of endothelium, which may be a contributor to dysfunctional vasorelaxation observed in mesenteric arteries from post-MI rats.

In addition, we also assessed the structure of the smooth muscle layer (data not shown). As expected, the structure of smooth muscle layer of mesenteric arteries from post-MI animals was comparable to Sham in terms of thickness of vascular wall, intra- and extracellular matrix, and distribution of fibers. These structural observations further confirm that dysfunctional vasorelaxation in mesenteric arteries from post-MI animals is mainly due to an impairment specifically in endothelium, but smooth muscle is unimpaired.

**MI attenuates the activation of eNOS-NO signaling.** eNOS-NO signaling plays a critical role in vasorelaxation, and its attenuation can result in dysfunctional vasorelaxation (22). To assess possible impairment of eNOS-NO signaling in mesenteric arteries from post-MI animals, we measured phosphorylation of eNOS at Ser1177. We first determined eNOS distribution in mesenteric arteries using immunohistochemical analysis and found that eNOS is mainly expressed in endothelium, consistent with previous studies (22, 24) (Fig. 3, A–C). As phosphorylation at Ser1177 is necessary for the maximal activation of eNOS and results in NO production, we measured phosphorylation of eNOS at Ser1177. As shown in Fig. 3, eNOS phosphorylation at Ser1177 was significantly reduced in mesenteric arteries from post-MI animals compared with Sham, and this decrease in eNOS phosphorylation was accompanied by decreased NO production. These results indicate that eNOS-NO signaling was impaired, suggesting that impaired eNOS-NO signaling may contribute to dysfunctional vasorelaxation in post-MI animals.

**MI attenuates the activity of PI3K-Akt signaling.** Our laboratory previously demonstrated that eNOS-NO signaling is mainly activated by upstream signaling from Akt, and that
inhibition of Akt or PI3K may significantly decrease the activity of eNOS and NO production (55). This suggests a possibility that decreased activity of eNOS observed in post-MI animals may be due to a decrease in PI3K-Akt signaling activity. To test this hypothesis, we assessed the activation of PI3K and Akt. As shown in Fig. 4, MI significantly decreased the activation of PI3K and Akt, as shown by the p-Akt-to-total Akt ratio and p-PI3K-to-total-PI3K ratio \( (P < 0.05–0.01) \), suggesting that MI caused a significant impairment in PI3K and Akt signaling in mesenteric arteries, which possibly contributes to decreased activation of eNOS-NO signaling.

**MI attenuates PI3K, Akt, and eNOS phosphorylation, and NO production induced by ACh.** Considerable evidence has demonstrated that eNOS-derived NO exerts a pivotal role in vasorelaxation, and PI3K and Akt are known to be important upstream regulators of eNOS activation (29, 34). The evaluation of vessels stimulated with ACh from each group could provide more convincing evidence that alteration of PI3K/Akt-mediated eNOS signaling pathways contributes to endothelial dysfunction. Therefore, we further studied protein phosphorylation in the presence of ACh in mesenteric arteries. Western blot analysis showed that PI3K, Akt, and eNOS phosphorylation by ACh were significantly decreased in mesenteric arteries from pos-MI animals compared with Sham (Fig. 5, A–C) \( (P < 0.05–0.01) \). These results suggest that PI3K/Akt-mediated eNOS signaling was attenuated in mesenteric arteries of post-MI animals, which may account for the impaired induction of vasorelaxation by ACh. We also investigated the effects of LY-294002 and L-NAME on ACh-induced NO production in mesenteric arteries (Fig. 5D). The results showed that ACh induced NO production, and this effect was reduced when mesenteric arteries were pretreated with LY-294002 or L-NAME. Although ACh increased NO production in MI mesenteric arteries, this effect was markedly weaker than in Sham \( (P < 0.01) \).

**Eight-week exercise improves cardiac function and normalizes endothelial structure and vasorelaxation.** Recent studies have shown that exercise can improve the function and structure of heart and vessels, and clinical observations also clearly indicate that patients with heart disease can significantly improve cardiac function and decrease related complications by
low-intensity aerobic exercise (22, 23). To further assess whether exercise may also decrease cardiac infarct size and improve cardiac function post-MI, MI rats were subjected to 8-wk treadmill exercise initiated 1 wk after surgery. As shown in Table 1, rats subjected to exercise did not show the reduced infarct size, although there was a trend toward reduced infarct size. In addition, exercise protocol used in this study also did not induce heart hypertrophy, as shown by the absolute and relative heart weight. However, exercise significantly increased body weight and decreased heart rate in MI/Ex compared with MI ($P<0.05$) (Table 1). More importantly, exercise for 8 wk significantly ameliorated cardiac dysfunction induced by MI, as evidenced by increased SBP, LVSP and $\frac{dP}{dt}$, and decreased LVEDP ($P<0.05–0.01$ vs. MI).

To determine whether exercise also ameliorated dysfunctional vasorelaxation induced by post-MI, dilation of mesenteric arteries was measured after 8-wk exercise. As shown by ultrastructural analysis (Fig. 2, D–I), degraded structure induced by post-MI was markedly less in exercised animals, suggesting that exercise after MI can restore cell integrity in mesenteric endothelial cells, which may be a potential mechanism for exercise-induced enhancement in vasorelaxation. Exercise training ameliorated post-MI-associated endothelial dysfunction, as evidenced by enhanced ACh-induced vasorelaxation in mesenteric arteries (maximal vasorelaxation: $86.0 \pm 5.2\%$ in MI/Ex vs. $66.1 \pm 5.4\%$ in MI; $P<0.05$; Fig. 1A), and the inhibition of either PI3K by LY-294002 (20 M given 15 min before stimulation) or eNOS by L-NAME (0.2 mM given 15 min before ACh stimulation) markedly inhibited exercise-restored vasorelaxation (maximal vasorelaxation: $23.8 \pm 4.9$ and $37.5 \pm 4.5\%$, respectively; Fig. 1C). In addition, endothelium-independent relaxation induced by SNP and constriction induced by CaCl$_2$ were not significantly different among the three groups (Fig. 1, E and F). These results indicate that ACh-induced vasorelaxation in mesenteric arteries was endothelium dependent and PI3K/Akt and eNOS mediated.

Eight-week exercise restores post-MI-associated deactivation of PI3K, Akt, and eNOS. Having demonstrated that exercise restored cellular integrity, we further assessed the possibility that exercise would also reverse the deactivation of PI3K, Akt, and eNOS induced by post-MI. We found that, following 8-wk exercise training, the activity of PI3K, Akt, and eNOS induced by post-MI. These results strongly suggest that MI causes a marked impairment in mesenteric arterial PI3K, Akt, and eNOS signaling in mesenteric arteries, and that exercise significantly reverses
post-MI-associated deactivation of PI3K, Akt, and eNOS. Taken together, these results indicate that depressed PI3K/Akt-mediated eNOS signaling is a critical mechanism, which is responsible for MI-associated dysfunctional dilatation of mesenteric arteries in MI rats, and that exercise reverses MI-associated dilatation dysfunction via the restoration of the activities of PI3K, Akt, and eNOS.

Eight-week exercise restores post-MI-associated loss of PI3K, Akt, and eNOS phosphorylation, and NO production in response to ACh stimulation. Previous studies have demonstrated that PI3K/Akt-mediated eNOS signaling cascades play a critical role in vasorelaxation induced by ACh (53). To determine whether exercise ameliorated MI-associated dysfunctional via a PI3K/Akt-mediated eNOS signaling, PI3K, Akt, and eNOS phosphorylation, and NO production induced by ACh were determined in mesenteric arteries (Fig. 5, A–D, respectively). Following 8-wk exercise training, PI3K, Akt, and eNOS phosphorylation, and NO production induced by ACh were significantly increased in MI + Ex mesenteric arteries ($P < 0.05$–$0.01$). These results suggested that MI causes a significant impairment of PI3K/Akt/eNOS signaling and that exercise markedly reverses MI-associated mesenteric arterial PI3K/Akt/eNOS signaling dysfunction.

DISCUSSION

In the present study, we have demonstrated that cardiac ischemia not only causes cardiac dysfunction, but also causes dysfunctional relaxation in mesenteric arteries, likely secondary to degradation of endothelial structure, decreased activities of PI3K, Akt, and eNOS, and reduced the phosphorylation of PI3K, Akt, and eNOS, and subsequent NO production by ACh. Although this exercise protocol used in this study did not significantly reduce infarct size, exercise for 8 wk following cardiac infarction still improved cardiac function. Moreover, exercise for 8 wk following cardiac infarction not only restored endothelial structure, increased the activities of PI3K, Akt, and eNOS, but also elevated phosphorylation of PI3K, Akt, and eNOS, and NO production by ACh. This may, in part, account for the exercise-induced improvement of vasorelaxation. To our knowledge, this is the first study to comprehensively investigate the beneficial effects of exercise on endothelial ultrastructure, vascular function, and related mechanisms in post-MI animals.

Recently, numerous attention and effort have been focused on ischemic myocardium and endothelial dysfunction in the area of an MI, given that this is directly associated with cardiac dysfunction and resultant heart failure (18, 32). Endothelial dysfunction is generally characterized by reduced synthesis or altered activity of vasodilative mediators and increased effects of vasoconstrictors (36). However, it is relatively unknown whether other vessels, particularly mesenteric arteries, also display post-MI-associated dysfunctional changes. Mesenteric arteries are an important constituent of peripheral resistance vessels that harbor approximately one-fifth of total blood volume and have been implicated in the regulation of both systemic blood pressure and circulating blood volume. In the present study, we observed dysfunctional relaxation of mesenteric arteries in post-MI animals, as shown by decreased ACh-induced vasorelaxation, suggesting that MI not only causes dysfunctional vasorelaxation within the infarcted area, but also induces dysfunctional vasorelaxation in peripheral resistance vessels, such as mesenteric arteries. These pathological changes are characterized by impairments in endothelial integrity and vasorelaxation, consistent with previous studies in ischemic coronary arteries (24). The smooth muscle layer did not display discernible changes in ultrastructure and function.

eNOS-derived NO production in endothelium has been shown to play a critical role in vasorelaxation, because the deletion of eNOS or decreased eNOS activity causes dysfunctional vasorelaxation in most vessels. Under normal conditions, a major component of the relaxation response is mediated by endothelium-derived hyperpolarizing factor, but, after MI, NO-dependent relaxation becomes dominant (49). Our laboratory’s previous study conducted in coronary arteries subjected to ischemia-reperfusion demonstrated a cause-effect relationship between dysfunctional vasorelaxation and decreased eNOS-NO activation (24). In the present study, we further observed that post-MI-associated dysfunctional vasorelaxation in peripheral resistance arteries was associated with decreased eNOS-NO signaling, suggesting the existence of a common mechanism of...
dysfunctional vasorelaxation. In addition, we also showed that post-MI also decreased the activation of the serine/threonine protein kinase, Akt/PKB, and its upstream mediator, PI3K, a phenomena present in ischemia-reperfusion coronary arteries (24). Decreased activity of the PI3K-Akt pathway may account for decreased eNOS-NO activation, as suggested by the fact that the decrease in Akt or PI3K activation decreases eNOS-derived NO production (8, 19, 29, 34). We found that MI may induce dysfunctional vasorelaxation in ischemic coronary arteries and peripheral resistance arteries by the same mechanism-decreased PI3K-Akt-eNOS-NO signaling. This was surprising because the microenvironments are markedly different between ischemia-reperfusion coronary arteries and post-MI peripheral resistance arteries. How may post-MI deactivate PI3K-Akt-eNOS-NO signaling in peripheral resistance arteries? Previous studies showed that inflammatory cytokines are released into blood from MI tissue, and superoxide anion production is increased post-MI, which impair endothelial function (1, 20, 27, 39, 47a, 54). In addition, angiotensin, norepinephrine, arginine vasopressin, and endothelin are also increased post-MI, blunting the activity of eNOS and increasing vasoconstriction (36). In addition, the decreased mesenteric blood flow may be a possible risk factor for the deactivation of the PI3K/Akt/eNOS pathway in peripheral resistance arteries after MI (4, 6, 15).

Exercise has been reported to ameliorate cardiovascular dysfunction in patients with cardiovascular disease (23, 25, 43, 44) and in animal models of cardiovascular diseases (5, 13, 22). However, the mechanisms by which exercise improves cardiovascular function are not completely characterized. Our laboratory’s previous study in aged vessels showed that exercise improved vasorelaxation by upregulating eNOS expression and increasing eNOS phosphorylation in response to insulin stimulation (22). Here, we further demonstrate that exercise can also protect endothelial structural integrity and improve vasorelaxation in response to ACh. This improvement in vasorelaxation may be closely associated with endogenous eNOS-NO signaling. Specifically, mesenteric arteries from post-MI animals show vascular dysfunction and decreased eNOS activation and NO production, and exercise may restore vascular function and increase eNOS activation and NO production, suggesting a cause-effect relationship between de-

Fig. 5. Effects of MI and exercise training for the PI3K/Akt/eNOS signaling on mesenteric artery. The bar graphs below the blots show statistical results. The concentration of ACh was 10^{-5} M. LY-294002 (LY; 20 μM) and L-NAME (0.2 mM) were added to perfusate 15 min before the experiments. Values are means ± SE; n = 6 per group. ∆ P < 0.05, ∆ ∆ P < 0.01 vs. Sham mesenteric arteries receiving vehicle. ▽ ▽ P < 0.01 vs. Sham mesenteric arteries receiving ACh. •• P < 0.05, ••• P < 0.01 vs. MI mesenteric arteries receiving vehicle. •••• P < 0.01 vs. MI mesenteric arteries receiving ACh. ■ P < 0.01 vs. MI+Ex receiving vehicle. ★★★ P < 0.01 vs. MI+Ex receiving ACh.
increased eNOS-NO signaling and vascular dysfunction. We also demonstrate that exercise can improve PI3K-Akt activation, which is impaired post-MI, and which plays a key role in exercise-induced improvement of endogenous eNOS-NO signaling. Therefore, the interventions that activate the PI3K-Akt-eNOS signaling pathway in endothelial cells may have therapeutic benefits for post-MI-associated complications.

During exercise training, the increased cardiac output and subsequent blood flow may also be a potential factor for improving mesenteric arterial function, given that increased blood flow can produce shear stress in the mesenteric arteries, which has been shown to promote the expression and activities of PI3K, Akt, and eNOS and thus increase NO production in vivo or in vitro (7, 11, 15, 17, 42). In addition to Akt-induced eNOS phosphorylation, exercise-induced mitogen-activated protein kinase activation and upregulation of heat shock protein (HSP) 70 may also contribute to upregulated eNOS activation (21). A combination of HSP70 and HSP90 has been shown to potently induce eNOS expression. Importantly, HSP90 can rapidly bind to the eNOS molecule in response to stimulation, thereby enhancing its catalytic activity and subsequent eNOS-derived NO production (41). Although exercise has been demonstrated to upregulate HSP90 expression (14), further study is warranted to elucidate the contribution of HSP90 to the exercise-induced activation of eNOS in post-MI mesenteric arteries.

The effects of exercise training are not unique or specific to MI. Previous studies along with our studies showed that exercise training could increase the endothelial function in normal subjects, as well as abnormal subjects, such as aging, hyperlipidemia, hypertension, or diabetes (5, 15, 22, 37, 46), and exercise improved endothelial function independently of the type of training (43). In addition, exercise training could increase the expression and phosphorylation of PI3K, Akt, and eNOS in animal’s vascular (15, 55). Collectively, the beneficial effects of exercise are multispatial. However, it is noteworthy that the beneficial effects of exercise are absolutely dependent on the product of exercise intensity and exercise time. Further study is warranted to elucidate the roles of other signaling transductions in exercise-induced improvement of endothelial function, such as adenosine monophosphate-activated protein kinase, which has been activated during exercise.

In summary, MI causes dysfunctional vasorelaxation in mesenteric arteries, possibly by degradation of endothelial structure and attenuating the PI3K-Akt-eNOS signaling cascade. Exercise improves vasorelaxation function by protecting endothelial structural integrity and activating the PI3K-Akt-eNOS signaling cascade. Collectively, our finding should shed some light on our understanding of post-MI-associated dysfunctional vasorelaxation and its reversal by exercise and may help to identify a new therapeutic regimen for post-MI-associated cardiovascular diseases. Our data also suggest that lifestyle interventions, such as physical activity, that improve endothelial function of mesenteric arteries have significant value in the prevention and treatment of dilatation dysfunction and its complications in post-MI patients.

ACKNOWLEDGMENTS

We are grateful to Professor Tzyh-Chang Hwang from the University of Missouri–Columbia for editorial assistance.

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GRANTS

This work was supported by grants from the National Natural Science Foundation of China (Key Program, no. 30930105; General Program, nos. 30873058, 30707785), the National Basic Research Program of China (973 Program, no. 2007CB512005), and China Medical Board Distinguished Professors Award (no. F510000/G16916404).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).


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