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Enhanced external counterpulsation improves endothelium-dependent vasorelaxation in the carotid arteries of hypercholesterolemic pigs

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Abstract

Background: Enhanced external counterpulsation (EECP) has been demonstrated to be an effective method for the treatment of atherosclerotic vascular disease. However, the exact mechanism underlying the beneficial effects of EECP is not completely clear. We hypothesized that EECP leads to improvement in endothelial function, contributing to its clinical benefits.

Methods: Fifteen male domestic pigs were initially divided into 2 dietary groups: one consumed a normal feeding (NF) of pig chow ($n=5$), and one consumed a high-fat (HF) pig chow ($n=10$). After 8 weeks on the NF or HF diet, 5 HF pigs received EECP treatment (HF+EECP) 1 h daily for 6 weeks and the remaining 5 HF pigs continued to be fed by high cholesterol diet. At the end of 6-week EECP treatment, the carotid arterial rings from all of the pigs were harvested. Endothelium-dependent and -independent vasorelaxation to acetylcholine (ACh) and sodium nitroprusside (SNP) were measured in a dose-dependent manner.

Results: The high fat diet resulted in increase in plasma cholesterol and triglyceride levels ($p<0.05$). Endothelium-dependent vasorelaxation was decreased in the HF group compared to the NF control ($p<0.05$). However, EECP treatment partially improved impaired endothelium-dependent vasorelaxation in the HF+EECP group compared to the HF control ($p<0.05$). Endothelium-independent vasorelaxation was not significantly different among the three groups.

Conclusions: Endothelium-dependent vasorelaxation is impaired in the hypercholesterolemic pigs. EECP treatment significantly improves hypercholesterolemia-induced diminished endothelium-dependent vasorelaxation. It suggests that amelioration in endothelial function may at least in part contribute to the beneficial effects of EECP treatment in clinical practice.

Keywords: Endothelium; Hypercholesterolemia; Enhanced external counterpulsation; Endothelial function; Atherosclerosis

The endothelium, which is lined at the interface between flowing blood and vascular wall, transduces biological and mechanical stimuli within the circulation into physiological responses that regulates vascular homeostasis. Accumulating evidence indicates that vascular endothelial dysfunction plays a pivotal role in the pathogenesis of atherosclerotic vascular diseases [1–3]. The endothelium is involved in the initiation and development of atherosclerosis by producing and releasing a series of biological agents. One among them is nitric oxide (NO). NO has multiple beneficial effects in protecting the artery wall from cellular and lipid infiltration and preventing the endothelial surface from platelet aggregation and clotting. Many of the cardiovascular risk factors lead to impaired endothelial function and endothelial dysfunction due to NO deficiency contributes to the pathogenesis of atherosclerotic vascular disease including coronary artery disease [4–7]. Integrity of endothelial function may provide protection on the homeostasis of cardiovascular system. Therefore, improvement in endothelial function is a novel therapeutic strategy for the atherosclerotic vascular disease.

It has been demonstrated that some medications such as ACE inhibitor and statin lipid-lowering agents exhibit beneficial effects on the treatment of atherosclerotic vascular disease, which is at least in part related to improvement in endothelial function [8,9]. Studies also showed that enhanced external counterpulsation (EECP) is

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an effective method for the treatment of atherosclerotic vascular disease [10–12]. The EECP induces retrograde flow of blood from the lower extremities into the central aorta and produces a large diastolic pressure wave that augments coronary perfusion and flow in a manner similar with intra-aortic balloon pumping. Although the clinical efficacy of EECP treatment is achieved, the exact mechanism underlying the beneficial activities of EECP is not completely clear. We hypothesized that one of the underlying potential mechanisms of the beneficial activities using EECP treatment is related with improvement in endothelial function. Indeed, recent studies provided data to show that in patients with coronary artery disease EECP treatment improves flow-mediated vasorelaxation in the brachial artery and in parallel the clinical symptoms of angina pectoris are also reduced [13,14]. Until now there is no study to investigate effect of EECP on endothelium-dependent vasorelaxation in the hypercholesterolemic pig. To further test whether EECP therapy could improve endothelium-dependent vasorelaxation, we first established the hypercholesterolemic pig model which is demonstrated to have endothelial dysfunction in the vasculature and then studied effect of EECP treatment on NO-mediated, endothelium-dependent vasorelaxation in the carotid arteries of pigs with hypercholesterolemia.

1. Methods

1.1. Animals

All procedures involving animals were approved by the Animal Care and Use Committee of our University. The experimental animals were adult male domestic pigs ($n=15$) that were purchased from our hospital animal center. The pigs were 8–12 months of age and weighed 25–30 kg. All of the pigs were housed in a temperature-controlled room (20–22 °C) with a 12-h light/dark cycle. Five pigs consumed a normal feeding (NF) of chow diet. Another 10 pigs were fed a high-fat (HF) chow diet consisting of pig chow supplemented with cholesterol (4%), pig oil (8%), egg nuclear powder (10%), and sodium cholate (1.2%). After 8 weeks on the NF or HF diet, 5 HF pigs received EECP treatment (HF+EECP) 1 h daily for 6 weeks and the remaining 5 HF pigs continued high cholesterol diet feeding.

1.2. Lipid measures

Each pig had a baseline blood sample collected. The next sample was taken after consuming either the NF or HF for 8 weeks. A final blood sample was collected when the pigs were killed after the end of the 6-week EECP therapy. For total cholesterol or triglyceride measurement, plasma was assayed directly by standard enzymatic kit. For LDL and HDL lipid contents, fractions from each pig corresponding to these lipoproteins were collected and measured by standard enzymatic assay.

1.3. The EECP treatment

As shown in Fig. 1, the EECP device (Huawen, Inc., Fusung, China) is specifically designed for the present study and contains pumps and valves and reusable fabric cuffs, which are fastened around the pig's calves, thighs, and buttocks. During diastole, the cuffs are sequentially inflated first around the calves, then around the thighs, and finally around the buttocks, and are synchronized with the pig's electrocardiogram. The pressures applied to the cuffs are 0.04 mpa/cm^2. The full course of EECP treatment was 36 h with 1 h each day, extended over a 6-week period.

1.4. Endothelium-dependent and -independent vasorelaxation measurements

After the end of 6-week EECP treatment, All of the pigs were anesthetized with pentobarbital sodium (35 mg/kg iv) and the chest was opened to achieve euthanasia. The carotid arteries were rapidly excised and trimmed of connective and fat tissues for contractile tension recording. Vessel segments were taken from the same sites in all pigs.

Endothelium-dependent and -independent vasorelaxation were measured by standard arterial ring connected to force

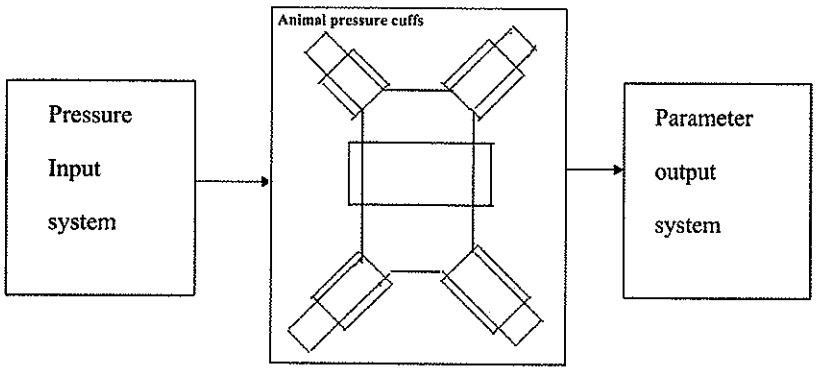

Fig. 1. Schematic device of pig experimental treatment with EECP.

transducers in a physiological bath. Arterial rings were mounted on tungsten wire triangles which was then connected to isometric force displacement transducers (FT03C, Grass Instruments Co., Quincy, MA). The rings were immersed in an oxygenased Krebs–Henseleit solution at 37 °C and allowed to equilibrate for 1 h under a 2-g preload. Tension was continuously recorded by an F-60 micro-displacement myograph (Narco Biosystems Inc., Houston, Tex). Before dose–response curves were initiated, all arterial rings were preconstricted with 10^{-6} mol/L norepinephrine to measure maximum contractile force. Endothelium-dependent vasorelaxation was assessed by using acetylcholine (ACh) 10^{-6} to 10^{-4} M. After the Ach dose–response curve was completed, the rings were washed with Krebs–Henseleit solution and allowed to equilibrate. They were subsequently constricted with norepinephrine as previously described. Endothelium-independent vasorelaxation was measured in response to sodium nitroprusside (SNP) 10^{-6} to 10^{-4} M.

1.5. Statistical analysis

All values are expressed as the mean±SD. Statistical analysis was performed using a SAS soft program. Unpaired Student *t*-test and one-way ANOVA were used to analyse the difference between groups. A p value of <0.05 was considered significant.

2. Results

There was no significant difference in body weight and blood pressure among the three groups at the end of EEECP treatment. The lipid files were shown in Fig. 2. The baseline fasting plasma total cholesterol and triglyceride levels, HDL and LDL were similar among the three groups. When the pigs were fed a HF diet for 8 weeks, LDL, HDL, and total plasma cholesterol and triglyceride levels were increased compared to the NF group ($p<0.05$). There was no difference in the lipid files between the HF+EECP group and HF group before and after 6-week EECP treatment (p=NS).

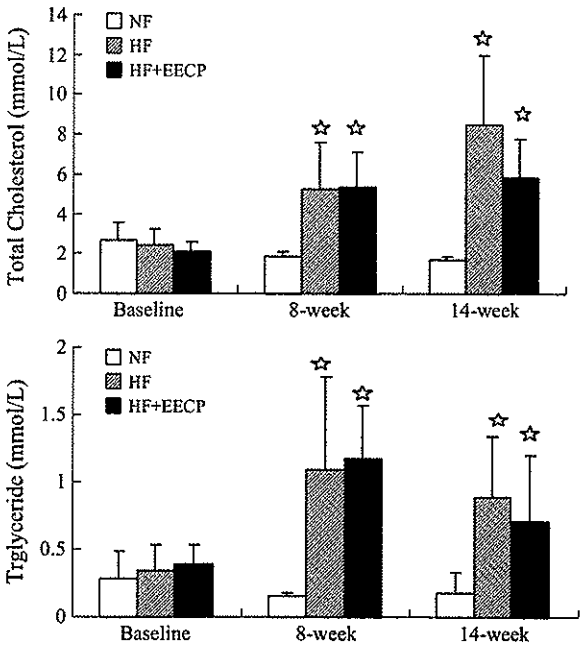

Fig. 2. Alterations in plasma lipid profiles among the three groups (☆$p<0.05$ compared with NF control).

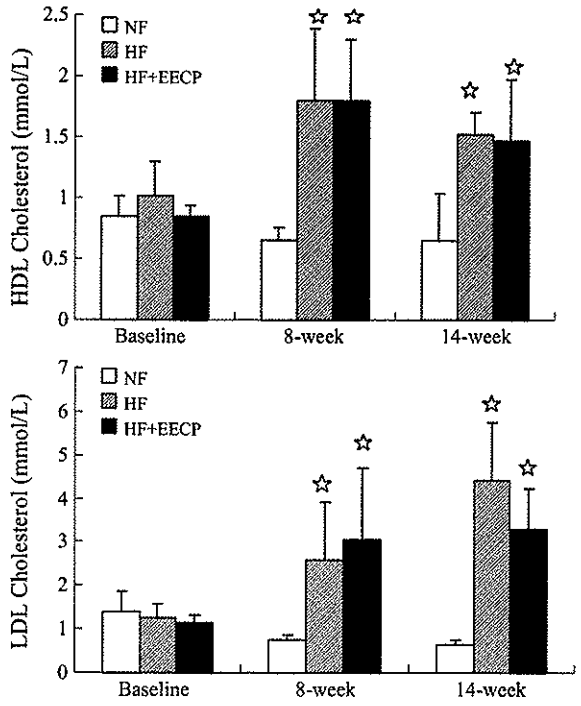

Fig. 3. Ach-induced vasorelaxation among the three groups (☆$p<0.05$ compared with NF control; $p<0.05$ compared with HF group).

The endothelium-dependent vasorelaxations in response to varying doses of ACh among the three groups were shown in Fig. 3. The endothelium-dependent vasorelaxations of the carotid arterial ring from the HF pigs were significantly reduced compared with those from the NF pigs as noted by vasorelaxation of 7±6% vs. 22±4% at 1×10^{-6} molar concentration ACh, 11±7% vs. 37±7% at 1×10^{-5} molar concentration ACh, and 17±10% vs. 54±2%, respectively ($p<0.05$). The endothelium-dependent vasorelaxations of the carotid arterial ring from the HF+EECP pigs were, however, significantly improved compared with those from the HF pigs alone as noted by vasorelaxation of 10±2% vs. 7±6% at 1×10^{-6} molar concentration Ach, 18±4% vs. 11±7% at 1×10^{-5} molar concentration ACh, and 27±6% vs. 17±10%, respectively ($p<0.05$).

The endothelium-independent vasorelaxations in response to varying doses of SNP were shown in Fig. 4. SNP elicited a concentration-dependent vasorelaxations in the carotid arterial rings from all groups. Direct smooth muscle vasorelaxations induced by SNP were similar among the three groups as noted by vasorelaxation of 21±1% vs.

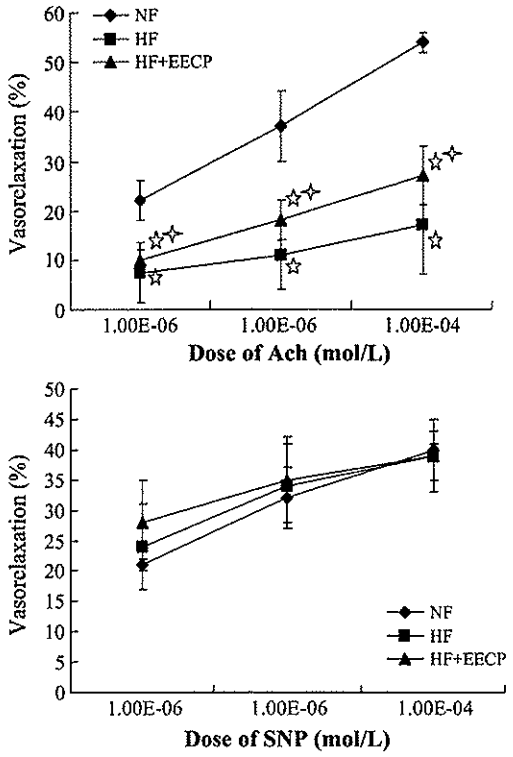

Fig. 4. SNP-induced vasorelaxation among the three groups.

24±7% vs. 28±7% at 1×10^{-6} molar concentration SNP, 32±5% vs. 34±7% vs. 35±7% at 1×10^{-5} molar concentration SNP, and 40±1% vs. 39±6% vs. 39±4% at 1×10^{-4} molar concentration SNP, respectively (p=NS).

3. Discussion

The major findings of the present study were that hypercholesterolemic pig group had decreased NO-mediated, endothelium-dependent vasorelaxation, suggesting damage to the endothelium. EECP treatment significantly improved hypercholesterolemia-induced impaired endothelium-dependent vasorelaxation, indicating protection to the endothelium. There was no significant difference on endothelium-independent vasorelaxation among the three groups, showing no effect of EECP on smooth muscle function.

EECP has been used as a therapeutic modality for the atherosclerotic vascular disease. EECP treatment produces an acute hemodynamic effect that is similar to that produced by the invasive intra-aortic balloon pump. Three sets of cuffs on the upper thigh, lower thigh, and calves of each leg are inflated with compressed air during the diastolic phase of the cardiac cycle and are deflated in early systole. This rapid inflation and deflation raises diastolic aortic pressure, increases coronary perfusion pressure, and provides afterload reduction and enhances venous return with a subsequent increase in cardiac output. EECP has been demonstrated to provide symptom relief and improve long-term prognosis in patients with coronary artery disease [10,11]. EECP also increased exercise tolerance and prolongs time to 1-mm ST segment depression. Moreover, the prevalence of exercise-induced reversible perfusion defects by thallium scintigraphy decreased after EECP treatment [12]. These results suggest that EECP is a therapeutic method for the atherosclerotic vascular disease. The mechanism by which EECP treatment improves clinical symptoms in patients with coronary artery disease is, however, not fully understood. We hypothesized that improvement in NO-mediated, endothelium-dependent vasorelaxation by EECP contributes to its clinical benefits.

To study effect of EECP on endothelial function, we chose the hypercholesterolemic pig as a model of endothelial dysfunction. One reason for this purpose is based on that pigs possess vascular system very similarly to that of humans. The other is that previous studies demonstrated that endothelial function is impaired by hypercholesterolemia in coronary and peripheral arteries and the vascular dysfunction induced by hypercholesterolemia is associated with blunted NO-mediated, endothelium-dependent vasodilator response to ACh, indicating that the hypercholesterolemic pig is an ideal model for evaluating effect of EECP treatment on endothelial function [15,16]. As shown in our present data, hypercholesterolemia leads to impaired ACh-induced vasorelaxation of the pig carotid arteries by diminished NO-mediated, endothelium-dependent vasodilatation, consistent to previous studies [15,16].

In the present study we determined whether EECP could improve endothelial function, and we hypothesized that EECP is able to augment NO-mediated, endothelium-dependent vasorelaxation and preserve endothelial function in the carotid arteries from the hypercholesterolemic pigs. As shown by the current study, EECP improved ACh-induced endothelium-dependent vasorelaxation, but SNP-induced endothelium-independent vasorelaxation kept unchanged in the hypercholesterolemic pigs with and without EECP treatment compared with control group, suggesting that the protective effect of EECP on cardiovascular system is, at least in part, related to amelioration of endothelial function. Indeed, recent studies showed that in humans EECP therapy improves flow-mediated endothelium-dependent vasorelaxation in the brachial arteries, supporting further our data of the present observation [13,14].

It should be pointed out that there are some limitations for the present study. First, although we demonstrated that EECP improves endothelial function in the hypercholesterolemic pigs, the data reported here cannot permit us to clarify the mechanism underlying the beneficial effect of EECP on endothelial function. It can be postulated that shear stress induced by chronic exposure to EECP is related to amelioration of endothelial function. Endothelial cells are constantly exposed to hemodynamic forces, which include the shear stress, the tangential force due to blood flow. Shear stress increases the mRNA level and the production of nitric oxide in endothelial cells' contributing to the maintenance of

the integrity of endothelial function [17–19]. Indeed, both animal study and clinical investigation from our laboratory have showed that EECP treatment increased plasma nitrate levels, an NO metabolite, and endothelial NO synthase expression, suggesting that EECP increases NO production [20,21]. We also found that in mongrel dogs EECP treatment reduces peripheral vascular resistance and this fall in peripheral vascular resistance is blocked by pretreatment with N^G-nitrio-L-arginine-methyl ester, a nitric oxide synthase inhibitor, further supporting that NO-mediated, endothelium-dependent vasorelaxation is preserved with EECP therapy (data not shown). Second, it has been shown that endothelium-derived hyperpolarization factor contribute to improvement in endothelial function related to shear stress [23,24]. This is beyond the present study and remains to be elucidated in the future investigation. Apart from the protective effect of EECP on endothelial function may be one of the mechanisms accounting for the salutary benefits of EECP, the other possible hypotheses are also proposed to explain its efficacy. These possible explanations include enhanced diastolic flow, changes in the neurohumoral milieu, changes in ventricular function independent of changes in cardiac load, and increased angiogenesis [24,25]. We think that all of the mechanisms mentioned above contribute to clinical benefits of EECP treatment. The exact mechanisms, which underlie beneficial effects of EECP treatment, remained to be further studied.

In summary, the present studies show that endothelium-dependent vasorelaxation was impaired by hypercholesterolemia in the porcine carotid arteries. The detrimental effect of the HF diet was characterized by diminished vasorelaxation to ACh due to reduced NO production. EECP treatment attenuated the deleterious effect of hypercholesterolemia on endothelial function by increasing NO-mediated, endothelium-dependent vasorelaxation, which may, at least in part, explain the beneficial activities of EECP treatment in clinical practice.

4. Uncited reference

[22]

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