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Inhibition of AAA in a rat model by treatment with ACEI perindopril

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ABSTRACT

Background: The purpose of the present study was to evaluate the effect of a new angiotensin converting enzyme inhibitor perindopril on the formation of experimental abdominal aortic aneurysms (AAAs) in a rat model induced by intraluminal elastase infusion and extraluminal calcium chloride ($CaCl_2$) application.

Materials and methods: Thirty-six male Sprague–Dawley rats were randomly distributed into three groups ($n = 12$ per group): model (A), sham (B), and perindopril (C). Rats in model and perindopril groups underwent intra-aortic elastase perfusion and extraluminal $CaCl_2$ application to induce AAAs. Rats in the sham group received aortic perfusion and extraluminal application of saline. A dose of 3 mg/kg/d of perindopril was fed orally in the perindopril group. The maximum abdominal aortic diameter was measured *in vivo* on days 0 and 28 and by ultrasound on days 7, 14, and 21. The arterial blood pressure was measured directly using a pressure transducer after cannulation in surgery and before death. AAA tissue samples were harvested at day 28 and evaluated using normal hematoxylin and eosin stain, Verhoeff-van Gieson stain for elastin, and image analysis technique.

Results: Aortic diameters of rats in the model group consistently increased within 28 d, coinciding with the development of a transmural inflammatory response, thickening of intima, and destruction of the elastic media. Without alteration in blood pressure, the AAA formation rate and mean maximal diameter of aorta at day 28 were significantly lower in the perindopril group compared with the control group (1.71 ± 0.20 *versus* 2.70 ± 0.69 mm, $P < 0.001$; 0% *versus* 90.91%, $P < 0.001$) and were similar to those in the sham group (1.79 ± 0.29 mm, $P = 0.175$; 0%, $P = 1$). The thickness of intima in the perindopril group was lower than that in the model group (20.68 ± 9.96 *versus* 58.49 ± 32.01 μm, $P = 0.001$), but higher than that in the sham group (7.23 ± 2.68 μm, $P = 0.005$). The intensity of elastin fiber showed the opposite trend (0.8541 ± 0.0495 in sham group *versus* 0.7376 ± 0.1024 in perindopril group *versus* 0.5413 ± 0.0912 in model group, $P < 0.001$).

Conclusions: Perindopril inhibited the aortic degeneration and AAA formation in the experimental AAA model induced by elastase and $CaCl_2$. This effect, which was independent of its influence on hemodynamics, appeared to be induced by the suppression of the inflammatory cell influx and intimal thickening and the preservation of aortic medial elastin.

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1. Introduction

Abdominal aortic aneurysm (AAA) is a potentially fatal disease, defined as a segmental dilatation exceeding 150% of the normal arterial diameter, with the risk for rupture increasing as the maximum diameter increases. The prevalence of the disorder is estimated at 3% in individuals >50 y, with male dominance [1]. 2.1% of all men older than 65 years died from AAA rupture. [2]. Even in the United States of America, despite improvement in the surgical treatment and perioperative care, AAA is among the 15 leading causes of death [3]. As patients with a large AAA, at least 5.5 cm in diameter, have an increased risk of rupture, elective surgical or endovascular repair is performed in these patients to prevent rupture [4]. Two large randomized trials reported that early elective surgical repair of small AAA did not improve the mortality rate [5,6]. It seems that patients and doctors are still waiting for aneurysms to rupture or to become large enough to justify surgical or endovascular repair [7]. Moreover, elderly patients with AAAs who have a poor quality of life because of a mental or physical handicap are not surgical candidates; therefore, the development of a noninvasive preventive or therapeutic approach for AAA is awaited.

AAA manifestations are characterized by acute and chronic inflammation of the vessel wall, matrix degradation, and vascular tissue remodeling [8]. Although elastin and collagen normally provide the resilience and tensile strength for the aorta, destruction of the elastic media and collagen degradation are considered necessary for aneurysm expansion and rupture [9]. Pharmacologic strategies aimed at preventing matrix degradation may therefore have the potential in the management of small asymptomatic AAAs [10].

Angiotensin converting enzyme (ACE) inhibitors are widely used to treat hypertension, congestive heart failure, and other cardiovascular diseases [11]. In addition to their vasodilator effects, these compounds are considered to have a substantial influence on connective tissue remodeling of heart and vascular wall [12,13]. In many circumstances, these effects arise through a direct modification of fibroproliferative tissue healing by inhibition of ACE in the vessel wall rather than the circulating enzyme alone [14,15]. Present studies about the potential effects of ACE inhibitors on aortic aneurysms are limited and controversial [16–19].

In this study, we investigated the effect of an ACE inhibitor (perindopril) on the process of experimental aneurysmal formation and matrix degeneration. Moreover, image analysis system was used for quantification analysis of the elastic media and thickness of intima.

2. Materials and methods

2.1. Animal and grouping

To avoid the influence of pregnancy in female rats and sex bias, only male rats were used for the experiment. A total of 36 pathogen-free male Sprague–Dawley rats aged 2 mo and weighing 250–350 g were provided by the Animal Experimental Center of West China Hospital, Sichuan University (License No. SCXK [Chuan] 2008-24). The protocol was approved by the Animal Ethics Committee of West China Hospital, Sichuan University. Animals were raised in a room maintained at 24°C–26°C and allowed free access to food and water. The rats were randomly divided into three groups ($n = 12$ for each group).

2.1.1. Model group

We used the rat model of a combination of intraluminal elastase infusion and extraluminal calcium chloride ($CaCl_2$) application. Tanaka *et al.* [20] have demonstrated that the success rate of AAA formation was significantly higher with a combination of intraluminal elastase infusion and extraluminal $CaCl_2$ exposure. In brief, a laparotomy was performed under sterile conditions, and the abdominal aorta was exposed from the level of the left renal vein to the aortic bifurcation. All lumbar branches of the exposed infrarenal aorta were ligated or clamped. The preperfusion aortic diameter (AD) was measured with a microvascular vernier caliper (W40380; Shanghai Medical Instruments, China) with an accuracy of 0.05 mm. A 24-ga catheter (BD intima II; Becton Dickinson Medical Devices, Shanghai, China) was introduced through a direct puncture at the aortic bifurcation, and its tip was positioned within the distal abdominal aorta. A pressure transducer (SCW MEDICATH, Shenzhen, China) was connected to the catheter; blood pressure (BP) was displayed on the monitor (G30; Philips, The Netherlands). After removing the pressure transducer, an altered sphygmomanometer (Yuyue Medical, Danyang, China) was connected to the catheter aimed at maintaining infusion pressure. Before clamping, heparin (0.5 mg/kg) was injected through the catheter to prevent thrombosis. An atraumatic microvascular clamp was placed on the aorta just below the left renal vein, and a temporary ligature with 4-0 silk suture was placed just above the aortic bifurcation to encompass the catheter without occluding it. The isolated segment of distal abdominal aorta (approximately 15 mm in length) was then perfused with 0.1 mL (about 5 U) of type I porcine pancreatic elastase (E-1250; Sigma Chemical, St Louis, MO), and a gauze soaked in 0.4 M $CaCl_2$ (E506; Amresco, Solon, IA) was wrapped around the aorta circumferentially. After 20 min of intraluminal static elastase infusion and extraluminal $CaCl_2$ exposure, the catheter was removed and puncture point of aorta was suture repaired. The postperfusion AD was measured 5 min after restoration of the aortic blood flow. Animals were allowed to recover from anesthesia and were maintained in individual cages with free access to food and water for as long as 28 d.

2.1.2. Sham group

The sham group consisted of 12 rats that underwent aortic perfusion and extraluminal application of saline. All animals were maintained in individual cages with free access to food and water for as long as 28 d.

2.1.3. Perindopril group

Twelve rats in the perindopril group were treated with intraluminal elastase infusion and extraluminal $CaCl_2$ application. A dose of 3 mg/kg of perindopril was fed orally on a daily basis from the perfusion day. The animals were raised under the same condition as others for 28 d.