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Downregulation of Remodelling Enzymatic Activity Induced by an Angiotensin-converting Enzyme Inhibitor (Perindopril) Reduces the Degeneration of Experimental Abdominal Aortic Aneurysms in a Rat Model

J.-M. Alsac [a,b,*], C. Journe [b], L. Louedec [b], J. Dai [b], P. Julia [a], J.-N. Fabiani [a], J.-B. Michel [b]

[a] *Service de Chirurgie Cardiaque et Vasculaire, Hôpital Européen Georges Pompidou, APHP, Université René Descartes, Faculté de Médecine, Paris 5, France*
[b] *Unité INSERM 698, Université Denis Diderot, Paris 7, France*

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Abstract *Aims:* Angiotensin-converting enzyme (ACE) inhibitors have proven their ability to affect vascular wall remodelling, in addition to their anti-hypertensive effects. The aim of this study was to assess the impact of perindopril on the development of abdominal aortic aneurysm (AAA) in a rat model, and its correlation to enzyme activities involved in vascular wall remodelling.
Methods: The model of the decellularised aortic xenograft in Lewis rat was chosen. Rats were randomised to two groups: group P fed with 3 mg kg^{-1} of perindopril daily during 30 days, or control group C ($n = 15$ per group)). Rats were euthanised at 30 days for analysis. AAA growth and histological changes in the aortic wall were measured by histomorphometry. Proteolytic activities were measured by gelatin zymography of conditioned medium for activematrix metalloproteinase 9/pro-matrix metalloproteinase 9 (MMP9/pro-MMP9) and activeMMP2/pro-MMP2, and by quantitative immunofluorescence tissue for elastase and plasmin.
Results: The mean maximal diameter of AAAs at 30 days was significantly lower in the treated group P compared with the control group C (2.5 ± 1.0 vs. 4.9 ± 2.1 mm; $P < 0.01$). The expansion rate of AAAs after 30 days was significantly reduced in group P compared with group C ($36 \pm 14\%$ vs. $67 \pm 23\%$; $P < 0.01$). Pro-MMP9 and MMP9 activities were significantly decreased in relative intensity (RI) in group P compared with group C (0.43 ± 0.64 RI vs. 1.02 ± 0.61 RI,

* Corresponding author. Service de Chirurgie Cardiaque et Vasculaire, Hôpital Européen Georges Pompidou, 20-40 rue Leblanc, 75908 Paris Cedex 15, France.
E-mail address: jean-marc.alsac@egp.aphp.fr (J.-M. Alsac).

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$P = 0.01$; 0.18 ± 0.57 RI vs. 0.66 ± 1.19 RI, $P = 0.004$). The activation rate of MMP2 was also significantly lower in group P compared with group C (1.27 ± 0.42 vs. 1.67 ± 0.44; $P = 0.002$). Elastase and plasmin tissue activities were significantly lower in group P compared with group C, respectively (3.9 ± 3.3 vs. 5.8 ± 3.7 IF min^{-1} g^{-1},and 25.9 ± 23.9 vs. 49.1 ± 38.7 IF min^{-1} g^{-1}; $P < 0.05$).

Conclusion: After 30 days of treatment by perindopril, a significant decrease in aneurysmal degeneration of the decellularised aortic xenograft AAA model was observed. This phenomenon appears to be induced by a downregulation of enzymes involved in the aortic wall remodelling during aneurysmal degeneration.

Abdominal aortic aneurysms (AAAs) are characterised by segmental dilatation of the aortic wall and pathologic remodelling of the extracellular matrix.[1] Although elastin and collagen normally provide the resilience and tensile strength needed for the aorta to withstand haemodynamic stress, destruction of the elastic media is an early event in aneurysmal degeneration, and collagen degradation is considered necessary for aneurysm expansion and rupture.[2] Experimental AAAs are associated with chronic transmural inflammation and elevated local expression of enzymes that mediate matrix protein degradation, including matrix metalloproteinases (MMPs), elastase and plasminogen activators.[3–6] Pharmacologic strategies aimed at preventing matrix degradation may therefore have promise in the management of small asymptomatic AAAs.[7] Angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of hypertension, congestive heart failure and other cardiovascular disorders.[8] Besides their vasodilatator effects, these compounds are recognised as having a substantial influence on connective tissue remodelling after myocardial infarction or vascular wall injury.[9,10] In many circumstances, these effects arise through a direct modification of fibroproliferative tissue healing rather than haemodynamic changes alone, and they may involve inhibition of ACE in the vessel wall, as opposed to the circulating enzyme alone.[11,12] Despite the widespread use of ACE inhibitors in patients with cardiovascular disease, little is known about their potential effects on aortic aneurysms. A previous study by Liao et al.[13] demonstrated that ACE inhibitors have an important influence on experimental aneurysmal degeneration, but that these effects are distinct from changes in systemic haemodynamics alone or events mediated solely by type 1 angiotensin II (ANG II) receptors. However, their molecular, cellular and physiologic mechanisms remain to be elucidated.

In this study, we investigated the effect of a treatment by an ACE inhibitor (perindopril) on the process of experimental aneurysmal degeneration, and its correlation with enzymatic activities involved in aortic wall remodelling.

Methods

Preliminary control study design

Fifteen Lewis rats normotensive without AAA were used for a preliminary control study to determine the effect of the treatment by perindopril (3 mg kg^{-1} day^{-1}) during 1 month on systemic haemodynamics and to show that ACE activity was efficiency-inhibited at the dosage used in this particular model.

Haemodynamic measurements

The systolic blood pressure (SBP) was measured using the tail–cuff method (IITC Life Science), previously described,[14] in all 15 rats. Six individual recordings were made in a 30-min period of stabilisation, and the mean of these values was used to establish the pre-treatment baseline conditions for each animal. Haemodynamic measurements were repeated with the same technique after 30 days of perindopril treatment.

Renin activity

One millilitre of blood was sampled on citrate at the time of anaesthesia at day 0 and after 30 days of treatment by perindopril. Blood samples were centrifuged (1500 rpm for 10 min), and plasma recuperated for analysis of plasma renin activity (PRA). PRA was measured by radioimmunoassay of the angiotensin I (ANG-I) produced by incubation of plasma at 37 °C for 1 h.[15]

Study on experimental AAA models

We used the model of the decellularised aortic xenograft in rat, as previously described.[16,17] Animals were fed a standard diet. Animal care complied with the principles of laboratory animal care formulated by the European Union, and experimentation was carried out under the authority of the French Agriculture Ministry (authorisation n75-214, delivered 25 March 2003).

Graft preparation

The xenografts were abdominal aortas from male guinea pigs (body weight (b. w.) 350 g, centre Ardenay, France). Guinea pigs were anaesthetised with pentobarbital (5 mg 100 g^{-1} b.w. intra-peritoneally) and a midline laparotomy performed. A 1-cm-long segment of infrarenal aorta was removed after ligature of collaterals under an operating microscope and rinsed with 0.9% saline solution. The guinea pig aortas were then incubated for 18 h at 37 °C in 0.1% sodium dodecyl sulphate (SDS) buffer with gentle agitation. The SDS-treated aortas were then washed once with Triton X-100, 0.1% in phosphate-buffered saline (PBS) for 24 h, and 4 times in PBS for 24 h. Decellularisation of the graft, using this technique, has been verified by examination of fixed sections with light and electron microscopy.[16]