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Original Article

Expression of vascular endothelial growth factor and receptor tyrosine kinases in cardiac ischemia/reperfusion injury

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Abstract

Introduction: Vascular endothelial growth factor (VEGF) expression is regulated by hypoxia and cytokines, including insulin-like growth factor (IGF)-1. We examined the influence of ischemia/reperfusion (I/R) on IGF-1, VEGF, fetal liver kinase (flk-1), fms-like tyrosine kinase-1 (flt-1), and laminin using an isolated hemoperfused working porcine heart model of acute ischemia (2 h) and reperfusion (4 h). **Methods:** Twenty-three porcine hearts were randomized into the following groups: five nonischemic control hearts (Group C), five I/R hearts with occlusion of the ramus circumflexus; three I/R hearts treated with quinaprilat, a potent angiotensin-converting enzyme (ACE) inhibitor (Group Q); five I/R hearts treated with angiotensin I (Group Ang I), and 5 I/R hearts treated with Ang I and quinaprilat (Group QA). **Results:** Compared to C, VEGF mRNA and protein contents were significantly increased in I/R and Ang I hearts. flk-1 and flt-1 were increased in I/R (2.2-/1.95-fold) and further elevated by Ang I (3.2-/3.4-fold) compared with C. Quinaprilat application attenuated the amount of VEGF significantly and of flk-1 slightly but not that of flt-1. In contrast, IGF-1 and IGF-1 receptor (IGF-1R) proteins were elevated in I/R hearts (3-/1.4-fold vs. C) and further increased in the presence of Q. These findings were accompanied by an elevation of laminin mRNA and protein levels. Moreover, we observed an increase in collagen Type IV and chondroitin sulfate content in I/R (2.9-/1.4-fold) and Ang I (3.5-/1.5-fold) hearts. Quinaprilat significantly reduced laminin and chondroitin sulfate proteins. **Conclusion:** These data suggest that the VEGF/VEGF receptor and IGF-1–IGF-1R systems are activated by I/R. The benefits of ACE inhibition in attenuation of cardiac remodeling may be mediated by IGF-1.

Keywords: VEGF; IGF-1; Receptor; Myocardium; ACE-inhibition; Ischemia; Reperfusion

1. Introduction

The renin-angiotensin system (RAS) is very important for the regulation of systemic blood pressure and homeostasis. After myocardial infarction (MI), the RAS is activated and plays a key role in post-MI remodeling and left ventricular (LV) dysfunction. Inhibition of angiotensin-converting enzyme (ACE) has exerted beneficial effects post MI. There is increasing evidence that the RAS is involved in the up-regulation of vascular endothelial growth factor (VEGF) and angiogenesis [1–3]. VEGF is a specific endothelial cell mitogen and plays an important role in myocardial angiogenesis and vascular leakage. VEGF is significantly increased in chronically ischemic myocardium [4,5] and immediately up-regulated after coronary occlusion in the heart [6,7]. VEGF stimulates endothelial cell

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proliferation and acts via two receptors, the tyrosine kinase receptor fetal liver kinase (flk-1) and the tyrosine kinase receptor fms-like tyrosine kinase-1 (flt-1) [8], which are expressed by endothelial cells [9]. The effects of the RAS on the receptor/ligand systems flk-1/flt-1 and their ligand VEGF are not completely understood.

Elucidating the mechanisms that mediate cardiac tissue remodeling is very important, considering the fact that hypertrophy and an abnormal accumulation of extracellular matrix material in the interstitium has been associated with changes in LV function and heart failure. We recently reported that osteopontin and fibronectin were increased in early stages of MI [10]. Of the extracellular matrix proteins likely to play a role in the cardiac repair process after injury, laminins are of particular interest. Laminins are heterotrimers of alpha-, beta-, and gamma-chains linked by disulfide bonds to form a cruciform molecule [11–13]. Laminins are major components of basement membranes that mediate cell adhesion and generate differentiation signals when bound to their surface receptors [14]. Studies on wound healing have revealed that laminin plays a key role in the healing process. Laminin and Type IV collagen contribute to the extracellular matrix assembly post MI, but little is known concerning the early phases post MI.

Insulin-like growth factor (IGF)-1 appears to play an important role in cardiac hypertrophy or remodeling, but little is known about its effects in ischemia/reperfusion (I/R). It is known that IGF-1 is produced and released mainly from cardiac fibroblasts and that endogenous IGF-1 promotes collagen synthesis by cardiac fibroblasts and hypertrophy of myocytes as an autocrine and paracrine factor [15]. It was shown that IGF-1 is effective in stimulating the production of the extracellular matrix (ECM) components that accumulate in the mesangial region during the course of diabetic glomerular disease [16]. A recent paper reported that IGF-1 mediates mesangial cell cycle progression, hypertrophy, and ECM protein synthesis such as laminin [17]. This is a finding that may also play a role in ischemic heart disease.

Therefore, the principal aim of this study was first to investigate the effects of I/R on the growth factors IGF-1 and VEGF and their respective receptors in the presence and absence of the ACE inhibitior quinaprilat, which is the active metabolite of quinapril. Our objective was to find differences in their actions and in their possible impact on the changes of the ECM proteins chondroitin sulfate, laminin, and collagen Type IV.

2. Materials and methods

2.1. Organ selection and transport

Twenty-three porcine hearts were obtained from a slaughterhouse. Health conditions of animals were controlled and verified by a veterinarian. Only female German landrace pigs with a total body weight range of 60–80 kg were chosen and killed according to the rules established by the Veterinary Council of the European Community. Immediately after bleeding, sternotomy was performed and the heart was carefully excised and placed in cardioplegic solution, as published earlier [10,18]. The organs were transported to the laboratory within 1 h at 4°C.

2.2. Organ perfusion

The hearts were prepared and connected to the perfusion apparatus developed in collaboration with Mediport Biotechnik GmbH (Berlin, Germany) exactly as described earlier [10,18]. When connected, the hearts were warmed up to 37°C and stimulated to beat by starting the perfusion apparatus. This machine pumped autologous blood, which had been collected from the respective donor at the slaughterhouse, diluted 2:1 with a modified Krebs Henseleit solution [18], warmed up to 37°C by a heat exchanger connected to a water bath, and oxygenated by a flow meter (Krohne DK800R, Duisburg, Germany) with a mixture of oxygen and carbon dioxide (95% O_2 and 5% CO_2) through the organ.

2.3. Ischemia and reperfusion

When the hearts beat regularly after one initial hour of perfusion, 18 hearts were infarcted by occluding the ramus circumflexus of the left coronary artery for 2 h before reperfusion was continued for another 4 h (Group I/R, n=5); in parallel, five hearts were randomized to nonischemic controls (Group C, n=5). The ramus circumflexus was closed by a three-way valve. The perfusion was stopped, but it was possible to apply drugs such as angiotensin I (Ang I) and quinaprilat via the valve. After 2 h of occlusion, the vessel was opened again, and the heart was reperfused for 4 h (reperfusion time). Ten hearts were treated with Ang I (Sigma, Taufkirchen, Germany) injecting 100 μl 10^{-7} M Ang I in the ramus circumflexus. The dose was chosen according to Farquharson and Struthers [19]. Five of these hearts only received Ang I (group Ang I: n=5); the other five hearts received Ang I and quinaprilat (5 mg; Group QA: n=5). Three ischemic and reperfused hearts received quinaprilat without Ang I treatment (Group Q: n=3) [20,21]. Ang I and quinaprilat were applied for 30 min; the infusion started 10 min before the beginning of the reperfusion period.

2.4. Tissue samples

At the end of the perfusion experiment (after 4 h reperfusion time), the hearts were examined. The perfusion area of the occluded artery (area at risk) was determined by carefully following the course of each vessel. The I/R area was determined according to Koyanagi et al. [22] by gross pathological examination and light microscopic investigation of the hearts. Then, the whole left and right ventricle was cut serially from apex to base. Samples for this study