Peer reviewed article

Myocardial angiogenesis induction with bone protein derived growth factors (animal experiment)

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Summary

Myocardial angiogenesis induction with vascular growth factors constitutes a potential strategy for patients whose coronary artery disease is refractory to conventional treatment. The importance of angiogenesis in bone formation has led to the development of growth factors derived from bovine bone protein.

Twelve pigs (mean weight, 73 ± 3 kg) were chosen for the study. In the first group (n = 6, growth factor group) five 100 µg boluses of growth factors derived from bovine bone protein, diluted in Povidone 5%, were injected in the lateral wall of the left ventricle. In the second group (n = 6, control group), the same operation was performed but only the diluting agent was injected. All the animals were sacrificed after 28 days and the vascular density of the left lateral wall (expressed as the number of vascular structures per mm^2) as well as the area of blood vessel profiles per myocardial area analysed were determined histologically with a computerised system.

The growth factor group had a capillary density which was significantly higher than that of the control group: 12.6 ± 0.9/mm^2 *vs* 4.8 ± 0.5/mm^2 ($p < 0.01$). The same holds true for the arteriolar density: 1 ± 0.2/mm^2 *vs* 0.3 ± 0.1/mm^2 ($p < 0.01$). The surface ratios of blood vessel profiles per myocardial area were 4900 ± 800 µm^2/mm^2 and 1550 ± 400 µm^2/mm^2 ($p < 0.01$) respectively

In this experimental model, bovine bone protein derived growth factors induce a significant neovascularisation in healthy myocardium, and appear therefore as promising candidates for therapeutic angiogenesis.

Keywords: angiogenesis; therapeutics; myocardial ischaemia

Introduction

There is a growing number of patients who suffer from debilitating angina because of a coronary artery disease unresponsive to medication and who are neither candidates for percutaneous coronary angioplasty nor for coronary artery bypass surgery because the atherosclerotic lesions are too diffuse. Therefore alternative methods of treatment are currently under investigation. Among them, therapeutic angiogenesis appears to be a promising tool. Compensatory angiogenesis – the development of functional collaterals induced by specific growth factors – is a natural response to regional ischaemia. However, this process is rather slow and usually insufficiently compensates the ischaemia induced by an acute coronary artery occlusion. It appears therefore logical to bring growth factors directly to the ischaemic territories which are inaccessible to conventional treatment in order to amplify this compensatory mechanism.

The important role played by angiogenesis in bone formation [1, 2] gave the impetus to the development of growth factors derived from bone protein. This study was designed to analyse the angiogenic effects of these growth factors on healthy myocardium in a pig model.

Methods

Growth factor mixture

Bone protein (Provasc, Sulzer Carbomedics, Austin, Texas, USA) is the result of the isolation of proteins from bovine (< 2 years old, USA) femurs, marrow-free, in the range of 10 to 100 kDa. Based on ELISA assays some angiogenic factors have been detected such as FGF (fibroblast growth factor) and TGF-β (transforming growth factor-β). It is hypothesised that the mechanism of action is due to the synergy between multiple factors, some of which are not yet characterised. The growth factor mixture is diluted in 5% Povidone to a concentration of 1 mg/ml.

Animal preparation

The study was performed in 12 pigs (mean weight 73 ± 3 kg). The animals were premedicated with Ketamine (10 mg/kg) and Atropine (2 mg) injected intramusculary. Vascular access was established through a vein of the ear. After induction with sodium thiopentone (5 mg/kg) through this venous line, the animals were intubated and anaesthesia was maintained by intravenous administration of sodium thiopentone as needed. Three ECG leads were installed. A left lateral cervicotomy was performed to provide vascular access. A left lateral thoracotomy was performed through the fifth intercostal space. The pericardium was opened and reflected to form a cradle for suspending the heart.

Experimental protocol

The animals were randomised into two groups. In the first group (growth factor group, n = 6), five 100 µg boluses of bovine bone derived growth factor mixture were injected, 1 cm apart, in the lateral wall of the left ventricle. The injection sites were marked with a non-resorbable suture in order to localise them later for histology. In the second group (control group, n = 6), the same protocol was performed but Povidone solution without growth factor was injected. At the end of the operation, the thoracotomy was closed on a chest tube which was removed after weaning from the ventilator.

Control operation

After a month, the animals were sacrificed with an intravenous bolus injection of saturated potassium chloride and the hearts were rapidly excised for fixation in buffered formaldehyde (4%) for histology.

Histology and morphometry

The lateral wall of the left ventricle was excised and divided into two perpendicular to the axis of injection, at the mid-level of the myocardial thickness. Serial sections were stained with haematoxylin and eosin. The vascular endothelium was stained immunohistochemically with antifactor VIII (Dako, Glostrup, Denmark) using the Avidin-Biotin-Complex (ABC) Peroxidase method. The structures stained with factor VIII were counted with an image analysis system (Image Pro 3.0, Media Cybernetics, MD, USA) and expressed as the number of structures per mm^2. The area of blood vessel profiles was calculated in μm^2 and expressed per mm^2 of myocardial area examined histologically. For this analysis, three fields of 10 mm^2 were randomly selected within the marked areas of injection.

Statistics

The results of morphological analysis were given as mean plus or minus standard deviation. The vascular densities of the different areas were compared using a *t* test. Values were considered to differ significantly if $p < 0.05$.

Results

The growth factor group exhibited a vascular density which was significantly higher than that of the control group: 13.6 ± 0.5/mm^2 *vs* 5.1 ± 0.2/mm^2 ($p < 0.01$). When capillary and arteriolar structures were counted separately, the differences persisted: 12.6 ± 0.9/mm^2 *vs* 4.8 ± 0.5/mm^2 ($p < 0.01$) for the capillary density and 1 ± 0.2/mm^2 *vs* 0.3 ± 0.1/mm^2 ($p < 0.01$) for the arteriolar density. Blood vessel profile areas per myocardial area examined were 4900 ± 800 $\mu m^2/mm^2$ and 1550 ± 400 $\mu m^2/mm^2$ ($p < 0.01$) respectively. Figures 1 and 2 illustrate the difference of vascular density between both groups.

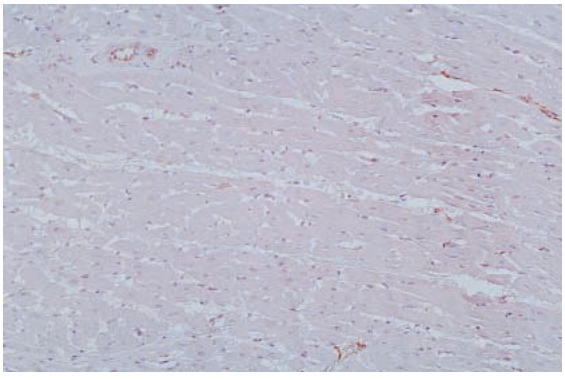

Figure 1

Factor VIII immunostaining of a sample of the control group (image width = 0.8 mm).

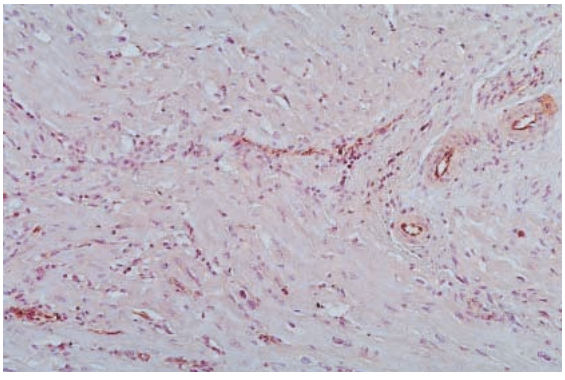

Figure 2

Factor VIII immunostaining of a sample of the growth factor group (image width = 0.8 mm). Both arteriolar or capillary densities are clearly increased when compared with figure 1.

Discussion

In this experimental model, bovine bone protein derived growth factors induced a significant neovascularisation, composed of both capillary and arteriolar structures, in healthy myocardium.

The idea for using bovine bone derived growth factor mixture came from the important role of angiogenesis in bone formation, including fracture healing [1] and endochondral ossification in the growth plate [2]. Murine osteosarcoma derived bone morphogenetic protein can induce ectopic bone through the whole process of endochondral bone formation. Yoshikawa et al. [3] have established an experimental model of induction of bone formation in which ectopic ossicles form reproducibly within three weeks of implanting collagen pellets containing bone morphogenetic protein into the back muscles of mice. These ossicles have been found to be highly vascular. In the same model [4], daily subcutaneous administration of TNP-470, a strong antiangiogenic agent, inhibited ectopic new bone formation in a dose-dependent manner. Histology revealed that TNP-470 prevented proliferation of mesenchymal cells and chondrogenesis at the initial step of endochondral bone formation and that this process resumed after discontinuing the TNP-470.

Individual growth factors have been shown to play an essential part in bone formation. When delivered directly into a freshly created fracture in the rabbit fibula, a single injection of bFGF and hyaluronan results in the stimulation of callus formation, increased bone formation and earlier restoration of mechanical strength at the fracture site [5]. Vascular endothelial growth factor (VEGF) inactivation results in a complete disruption of the normal vascular pattern of the growth plate of juvenile mice. Indeed, VEGF-mediated blood vessel invasion is essential for coupling resorption of cartilage with bone formation [6]. In this study, in order to produce only angiogenic effects without induction of bone formation, bone protein was injected in Povidone instead of supplying the matrix which is required for bone formation.

One potential advantage of using a mixture of multiple growth factors derived from bone protein lies in the fact that they may act synergistically. Two *in vitro* study [7, 8] have demonstrated that combined administration of VEGF and bFGF to endothelial cell cultures in three-dimensional collagen gels results in much greater and more rapid capillary tubule formation than the additive effects of either mitogen alone. Similar results were reported *in vivo* with the combination of VEGF and FGF [9] and that of SH/HGF and VEGF (scatter factor/hepatocyte growth factor) [10] in a rabbit model of hindlimb ischaemia. There is growing evidence therefore, that combinations of growth factors potentiate one another, and in that respect bone protein growth factor mixture has the potential to be a useful product even, perhaps, as a future treatment strategy in patients with ischaemic heart disease.

We wish to thank John P Ranieri and Lesley A Lakey from Sulzer Carbomedics (Austin, Texas, USA) and Monique Augstburger (Research nurse) for their contribution.

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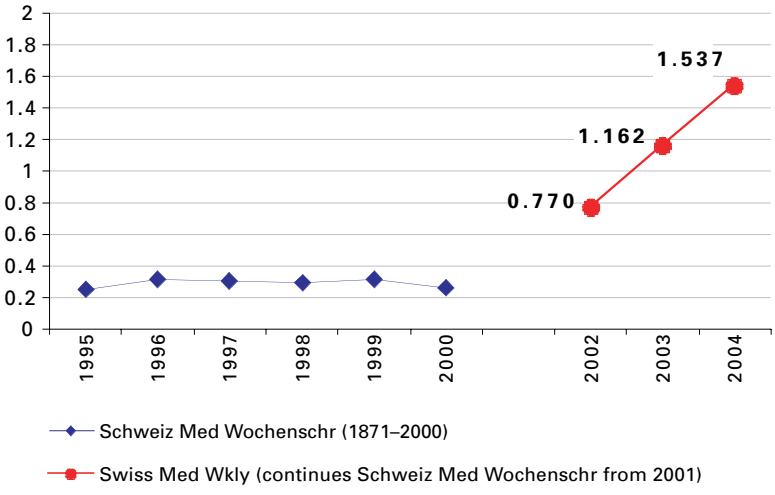

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