經導管轉植血管生長素基因及骨髓幹細胞進行治療性心肌血管新生

Therapeutic Myocardial Angiogenesis by Catheter-Based Transfer of Vascular Growth Factor Gene and Bone Marrow Stem Cells

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中文摘要

對於無法接受繞道手術或經皮冠狀血管擴張術的冠心症患者其治療目前仍有瓶頸。將載錄血管生成素的基因轉植入組織，以刺激血管新生（angiogenesis）的方法，已在許多動物心肌缺氧病變模型及人體實驗上有了令人振奮的結果。在植入基因的包裝方法包括質體（plasmid）或病毒載體（viral vectors）。在輸送途徑，則有經冠狀動脈灌流、心包膜內注射及直接注入心肌等途徑。而血管內皮前驅細胞（endothelial progenitor cells）在血管新生的重要性也漸為所知。如何產生具功能且持久的血管，無論是在血管生成素的種類、基因載入方法及運送途徑的選擇上，仍屬未定之論. 我們假設, 將載有 VEGF165 cDNA 的腺病毒及自體骨髓幹細胞經心導管術直接注入缺氧心肌，可強化側枝循環血管生成，改善局部灌流及心肌功能。更進一步對治療動脈硬化疾病的新要開發有所助益。

關鍵詞：冠心症、血管新生、血管內皮前驅細胞、基因療法

ABSTRACT

Therapeutic angiogenesis, in the form of growth factor protein administration or gene therapy, has emerged as a new horizon of the treatment of patients with severe, inoperable coronary artery disease. Improved myocardial perfusion and function after the administration of angiogenic growth factors or gene transfer, such fibroblastic growth factors (FGFs), vascular endothelial growth factors (VEGFs), has been demonstrated in animal models of chronic myocardial ischemia models. Angiogenesis by gene transfer is currently under investigation using a variety of growth factors, the way of gene transfer and a wide array of potential delivery system. These include application of gene as naked DNA, or by viral vector. The deliver systems include intracoronary, intrapericardial, direct intramyocardial techniques. The significance of endothelial progenitor cells in the enhancement of neovascularization is also promising. However, the optimal way to achieve the "functional and sustainable" angiogenesis is still unclear.

We propose that administration of replication-deficient adenovirus vector expressing VEGF165 cDNA in combination with vascular endothelial progenitor cells directly into ischemic myocardium which is defined by catheter-based electromechanical mapping would enhance collateral vessel formation, regional myocardial perfusion, and cardiac function.

Keywords: Coronary artery disease, angiogenesis, endothelial progenitor cells, gene therapy

BACKGROUND INTRODUCTION

Coronary heart disease is one of the leading causes of death in Taiwan. Classically, patients with coronary heart disease require a revascularization procedure, such as coronary artery bypass surgery or percutaneous transluminal balloon angioplasty. However, a large population of individuals with severe diffuse coronary artery disease exists for whom conventional therapies provide little or no benefit. It has been shown that incomplete revascularization is a predictor of a worsened outcome, including recurrent angina, myocardial infarction, congestive heart failure, or even death.

We have known for a long time that many patients with ischemic disease develop angiographically visible collateral vessels.[1] Recently, investigators have tested the hypotheses that the ischemia could be attenuated or abrogated by "therapeutic angiogenesis". Therapeutic angiogenesis, by stimulating the growth of new vessels that collaterize the affected vessel, in effect producing a "biologic bypass" - collaterization around a site of coronary occlusion, with concomitant improvements in regional myocardial perfusion and function, represents a theoretically attractive and intuitively rational new approach.[2]

In the last few years, clinical trails have been initiated with the goal of enhancing angiogenesis to treat peripheral vascular disease and ischemic heart disease. Although basic research in the field has provided several potential biologic agents to stimulating vessel growth, significant technical barriers remain in term of safe and practical local delivery.[3] An even more important issue is the function of new vessels. Some reports show the vessels formed by VEGF are leaky and tortuous. Whether this abnormal vascular morphology can lead to impair micro-circulation is not known.[4] Another outstanding question is whether a single angiogenic factor will be able to stimulate "functional and sustainable" angiogenesis.

Human gene therapy has been extensively studied for its potential to serve as an alternative method of modern medicine to treat inherited and acquired diseases. Through specific vehicles, such as liposome and viral derivative vectors, target DNA can be transferred to cells or tissue. Since 1993, first generation of the replication-deficient human subgroup C adenovirus have been evaluated in phase I trials for cancer and cystic fibrosis gene therapy.[5] In recent years gene therapy has become one of the most prospective and rapidly evolving field in biomedical and clinical research.

The investigation of angiogenesis in the heart is an exciting area of research, not only on the basis of scientific interest, but also in the potential for improving and reducing the cost of care of patients with ischemic heart disease. It is unlikely that therapeutic angiogenesis will significantly reduce the need for CABG or PTCA in the near future, but it has the potential to augment these treatment modalities and may be one of the treatment options to patients who are not candidates for conventional methods of myocardial revascularization.

RESULTS

Expression of $VEGF_{165}$ in HUVEC by Adenoviral Gene Transfer

To test whether VEGF can be expressed in endothelial cells by adenoviral (Ad) gene transfer, HUVEC cells were infected for 0 ~ 72 hours with a recombinant adenovirus containing the cDNA encoding $VEGF_{165}$ (Ad-$VEGF_{165}$). At each time point, total cell lysate was harvest and the expression of VEGF protein was detected by western blot analysis. As shown in figure 1, VEGF protein was first seen in the cell lysate 24 hours after infection; its expression increased with time and reached maximum at the 48^{th} hour. Furthermore, HUVEC cells infected with Ad-$VEGF_{165}$ survived longer in regular endothelial cell culture medium compared to those infected with Ad along.

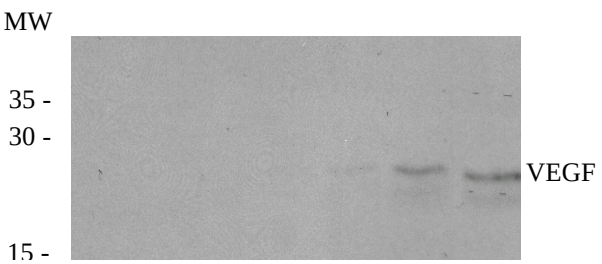

Figure 1. Western blot analysis of VEGF protein in the cell lysate of HUVEC infected with Ad-pGK (vector alone) for 72 hours (lane 1) or Ad-$VEGF_{165}$ for 0, 12, 24, 36, 48, and 72 hours (lanes 2-7).

Pig Model of Myocardial Ischemia

Chronic occlusion of coronary artery was induced in 6 miniswines with the use of an ameroid constrictor. Regional myocardial ischemia was seen by catheterization in all animals two weeks after the implantation of the constrictor. However, one animal had already developed collateral and two others died after catheterization. Consequently, only three miniswines were available for further manipulation.

Angiogenic Cell Therapy

After catheterization, all animals (n=3) underwent a midline sternotomy and autologous bone marrow derived mononucleated cells (BMN) (n=2) or normal saline (n=1) was injected directly into the ischemic myocardium. The presence of collateral in each animal was monitored by a second catheterization. Unfortunately, collateral development was seen in two animals, one injected with BMN and the other injected with normal saline.

DISCUSSION

In the present study, we have demonstrated that adenoviral vector is able to deliver exogenous genes into endothelial cells. Furthermore, $VEGF_{165}$ seemed to have preferential effect on the survival of HUVEC cells, which is consistant with the suggestion that autocrine endothelial VEGF contributes to the formation of blood vessels in a tumor and promotes its survival.[6] Because the endogenous VEGF is a secreted cytokine, it is necessary to investigate whether VEGF is also present in the conditioned medium from cell infected with Ad-$VEGF_{165}$.

Recent studies showed that transplantation of young bone marrow restores the pathways critical for cardiac angiogenesis in aging host.[7] In the present study, we tried to induce angiogenesis in ischemic myocardium with autologous bone marrow derived mononucleated cells. The initial results were not promising. However, the sample size was too small to make a conclusion. To improve our study, several things need to be determined in the future: (1) the best timing for initiating angiogenic cell or gene therapy, (2) the type and number of cells to be implanted, and (3) the route of administration.

SELF EVALUATION

In the last year, we had generated a protocol for adenoviral gene transfer and established a pig model of myocardial ischemia. It will take us more time to find out the conditions for combined angiogenic cell and gene therapies.

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