Repair effect of coexpression of the *hVEGF* and *hBMP* genes via an adeno-associated virus vector in a rabbit model of early steroid-induced avascular necrosis of the femoral head

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We investigated the repair effect of coexpression of the human vascular endothelial growth factor (*hVEGF*) and human bone morphogenetic protein (*hBMP*) genes via an adeno-associated virus (AAV) vector in a rabbit model of early steroid-induced avascular necrosis of the femoral head (SANFH). The following AAV vectors were constructed: AAV-green fluorescent protein, AAV-VEGF, AAV-BMP, and AAV-VEGF/BMP. The rabbit model was induced using lipopolysaccharide and methylprednisolone. Virus vector was injected into the core decompression region at a dose of 25 μL per side after core decompression operation in each group. $hVEGF_{165}$ and BMP-7 expressions were determined by Western blotting and immunohistochemical staining, and the femoral head was examined by magnetic resonance image scan, histopathologic staining, ink vessel staining, microcomputed tomography scan, and biomechanical assessment to determine the repair effect. The vector AAV-VEGF/BMP successfully expressed $hVEGF_{165}$ and BMP-7 at the gene and protein levels at 12 weeks after virus injection. The expression of $hVEGF_{165}$ promoted metabolism of the necrotic region by inducing vessel formation. The expression of BMP-7 promoted osteogenesis by increasing the mineral density and biomechanical strength of the femoral head. The repair effect of the AAV-VEGF/BMP group was better than those of the AAV-VEGF and AAV-BMP groups in the rabbit early SANFH model. The AAV-VEGF/BMP vector improved the bone repair capacity of the necrotic femoral head by inducing angiogenesis and improving bone quality in the femoral head. (Translational Research 2015;166:269–280)

Abbreviations: AAV = adeno-associated virus; BMD = bone mineral density; BMP = bone morphogenetic protein; GFP = green fluorescent protein; hrGFP = human recombinant green fluorescent protein; IOD = integral optical density; IRES = internal ribosome entry site; KD = kilodalton; Kg = kilogram; LPS = lipopolysaccharide; mg = milligram; mL = milliliter; MPS = methylprednisolone; MRI = magnetic resonance imaging; rAAV = recombinant adeno-associated virus; SANFH = steroid-induced avascular necrosis of the femoral head; SPF = specific pathogen free; μL = microliter; μg = microgram; vp = vector particles; VEGF = vascular endothelial growth factor

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AT A GLANCE COMMENTARY

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Background

Gene therapy has been increasingly recognized as a new therapeutic option for the treatment of avascular necrosis of the femoral head. Therapeutic neovascularization and bone formation is a promising approach for treating the disease. Adeno-associated virus is attractive and efficient for gene transfer in vitro and in vivo.

Translational Significance

The first in vivo experiment attempting to evaluate the repair effect of the coexpression of the human vascular endothelial growth factor and human bone morphogenetic protein on the steroid-induced avascular necrosis of the virus femoral head (SANFH) via an adeno-associated virus vector. Research aims to provide a solid foundation for the early treatment of SANFH via gene therapy, which eventually will become an effective treatment in the clinical practice.

Although there are some insights into the pathogenesis of avascular necrosis of the femoral head (ANFH) disease, there are no satisfactory methods to increase blood circulation in necrotic areas of the femoral head, to promote bone regeneration, or to prevent osteonecrosis. Gene therapy has been increasingly recognized as a new therapeutic option for the treatment of ANFH. Therapeutic neovascularization and bone formation is a promising approach for treating the disease. Among growth factors, vascular endothelial growth factor (VEGF) and bone morphogenetic protein (BMP) play important roles and have been studied extensively.

VEGF is one of the most important cytokines in angiogenesis. It promotes the division of vascular endothelial cells and induces angiopoiesis. VEGF is essential for bone formation and repair during the bone regeneration process, by directly attracting endothelial cells and osteoclasts, and enhancing the differentiation of osteoblasts.[1,2] BMP is the only signal molecule that can induce bone formation alone at orthotopic and heterotopic sites. BMP has definite effects on the stimulation of proliferation and the differentiation of mesenchymal and osteoprogenitor cells, as well as having efficient bone induction activity.[3,4] Because bone formation is a coordinated process involving BMP and VEGF,[5,6] orchestrating the presentation of these 2 factors may greatly enhance this process.

It is very important to choose a safe and effective vector system to transfer and correctly express a target gene during gene therapy. There are many natural features of adeno-associated virus that make the virus an attractive option for a human viral vector. It is the nonpathogenic defective human parvovirus that requires the presence of a helper virus, such as adenovirus or herpes virus, for efficient infection.[7,8] Other advantages of this vector system include its low immunogenicity, ability to transduce both dividing and nondividing cells, potential of integrating site specifically, ability to achieve long-term gene expression (even in vivo), and broad tropism allowing the efficient transduction of diverse organs.[9] All these features make adeno-associated virus (AAV) attractive and efficient for gene transfer in vitro and local injection in vivo.

We previously described the use of recombinant AAV (rAAV) vector as a gene transduction system for efficiently and stably coexpressing the $VEGF_{165}$ and *BMP-7* genes.[10] The expression of $VEGF_{165}$ and BMP-7 in bone marrow stem cells transfected with the AAV-VEGF/BMP vector enhanced angiogenesis and bone regeneration in vitro and in vivo. Here, rAAVs were directly injected into the defect region after decompression in a rabbit model of steroid-induced avascular necrosis of the femoral head (SANFH) to induce VEGF- and BMP-mediated comparative learning and promote neovascularization and bone regeneration in the necrotic region. The effects of AAV vector injection on repair were evaluated, and these data provide a new therapeutic option for SANFH.

The significance of this study is that this was the first in vivo experiment attempting to evaluate the repair effect of the coexpression of the human VEGF (hVEGF) and human BMP (hBMP) on the SANFH via an AAV vector. Our research aims to provide a solid foundation for the early treatment of SANFH via gene therapy, which eventually will become an effective treatment in the clinical practice.

MATERIALS AND METHODS

Animals. This study was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals, and all animal protocols were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University. All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Male New Zealand rabbits (license number, SCXK [Shaanxi] 2008-008; weight, 1.5–2.5 kg; age, 2 months; specific pathogen free class) were obtained from the Experimental Animal Center of Xi'an Jiao Tong University. The rabbits were bred and

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