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Vascular endothelial growth factor gene therapy with intramuscular injections of plasmid DNA enhances the survival of random pattern flaps in a rat model☆

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Summary The objective of this study was to determine the effects of the naked plasmid DNA encoding vascular endothelial growth factor (VEGF) on the survival of random flaps on rats. Thirty Sprague–Dawley rats whose random flaps were elevated on the back were randomised into three groups of 10 animals each. In the experimental group, the naked plasmid DNA encoding VEGF was injected directly into the panniculus carnosus of the flap. In the two control groups, either control plasmid DNA or physiologic saline was injected. After 7 days, the flaps were evaluated with the following devices: RT-PCR for the expression of VEGF gene, immunohistochemistry for the expression of VEGF protein, histology for vascular density, single photon emission computerised tomography for RBC in the flap, and image analysis for flap survival area. Notably increased expressions of VEGF mRNA and VEGF protein were found in the treatment group. Vascular density was markedly more increased in the treatment group than those in the two control groups ($P<0.01$). Compared with the two control groups, the flap treated with VEGF plasmid DNA showed a more significantly enhanced tissue viability: 87 ± 5 versus $47\pm6\%$ for the control plasmid DNA group and $46\pm5\%$ for the saline group ($P<0.01$). Our results

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indicated that the VEGF gene therapy was able to enhance the survival of random pattern flaps by inducing angiogenesis.

Plastic surgery has recently focused on the possibility that the viability of ischaemic tissues could be increased by gene therapy.[1,2] In an attempt to increase viability of ischaemic flaps, a number of growth factors that induce neovascularisation have been employed to treat ischaemic flaps and several of them, such as transforming-, fibroblast-, endothelial cell-, and vascular endothelial-growth factors, have demonstrated a marked ability to improve survival of the flaps.[3-6] Vascular endothelial growth factor (VEGF) is the most potent and specific one; its receptors are found solely on the endothelial cell.[7,8] Although the results were promising, the effects of VEGF protein could be transient because its half-life is approximately 30-45 min.[9] Therefore, gene therapy for ischaemic flaps has induced interests from scientists. Ischaemic skeletal muscle has been shown to be advantageous for taking up and expressing foreign genes transferred in the form of naked plasmid DNA.[10-15] The purpose of our study was to determine the effects of the naked plasmid DNA encoding $VEGF_{121}$, injected directly into the panniculus carnosus, on the survival of thick random pattern flaps in a rat model.

Materials and methods

Plasmids

The $pcD_2/hVEGF_{121}$, containing CMV (Cytomegalovirus) promoter, was kindly provided by Airu. Zhou, M.D. (Beijing Medical University). The cDNA encoding 121-amino acid isoform of human VEGF was cloned into Bam H I and Xba I of multiple cloning sites of the eukaryotic expressing vector pcD_2. The pcD_2 plasmid DNA without VEGF gene insertion was used for the control transfection experiments. The $pcD_2/hVEGF_{121}$ and pcD_2 plasmid were suspended in physiologic saline solution, respectively. The final working concentration of DNA for transference was 200 μg/ml.

Experimental protocol

Thirty female Sprague–Dawley rats weighing 280-320 g each were used in the study. The Animal Care and Experiment Committee of Jiangxi Medical College approved the experimental protocol. The animal model was established as previously described.[16] The rats were anaesthetised with a solution of intramuscular ketamine hydrochloride (30 mg/kg), and were randomised into three groups of 10 animals each. After the dorsal region was shaved, each animal was placed in a prone position, and a rectangle of 8 by 2 cm was drawn on the back of the rat. The 8 by 2 cm full thickness random pattern flap, consisting of skin, panniculus carnosus and fascial layers, was elevated by blunt dissection, with the pedicle remaining attached at the caudal end. Two different sites in each flap, which were 2 or 6 cm from pedicle with 1 cm from either lateral side, were chosen for subdermal injections. Each site was injected directly with $pcD_2/hVEGF_{121}$, or pcD_2 plasmid, or physiologic saline 200 μl solution, respectively, using a 1-ml syringe and 30-gauge needle. For each injection, the flap was turned over for the tip of needle to be inserted into the panniculus carnosus through the fascial layer, and the time of injection was slowed to approximately 6 s so that injected solution would not leak via the fascial layer. The first group was given the naked plasmid DNA encoding $VEGF_{121}$. A second group was given the control plasmid pcD_2 without the VEGF gene insertion, and a third group was given the physiologic saline alone. Following administration of the solutions, the flaps were returned to their recipient beds and carefully sutured into place with 3/0 silk sutures. The rats were returned to individual cages and fed for a period of 7 days.

Flap survival

At 7 days after the operation, the rats were placed in a prone position and flap survival areas were photographed and measured. Photographed images were scanned. The surface area of each flap and the respective area of necrosis were calculated with the colour medical image analytical system (CMIAS, Beijing Aerospace University and Air General Hospital, China). The percentage of survival region was measured by dividing the surviving area of the flap by its total surface area. Viable flaps were determined grossly, based on appearance, colour, and texture, etc.