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Abstract

Co-expression of VEGF and FGF2 mediated by multigenic plasmid constructs promotes blood flow restoration in a rat model of hind limb ischemia †

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Abstract: Peripheral arterial disease (PAD) is characterized by stenosis and occlusion of the arteries leading to the poor blood supply to the limb. Patients with PAD suffer pain at rest, intermittent claudication, skin ulcers, and gangrene. The end-stage of the disease could lead to limb amputation despite optimal medical and surgical management. The delivery of angiogenic factors to restore tissue perfusion is an attractive strategy, both as a primary and adjunctive treatment for PAD.
We synthesized multigenic plasmid constructs expressing combinations of VEGF, FGF2, and DsRed genes:pVax1-VEGF-FGF2-DsRed, pVax1-VEGF-DsRed, pVax1-FGF2-DsRed, pVax1-DsRed. In the constructed vectors, gene sequences are linked through the furin-containing 2A-peptide sequence of picornaviruses. Plasmid vector pVax1, approved by the FDA for use in clinical trials.
Previously, we confirmed the functionality of the developed non-viral constructs and the synthesis of VEGF, FGF2, and DsRed proteins by transfected cells. At this stage, we injected plasmid constructs into rat muscles after hind limb ischemia. Quantitative analysis of serum cytokines and chemokines of experimental and control groups on 3, 14, and 21 days after plasmids injection showed no significant differences in the secretion of the 18 cytokines studied. We observed a gradual increase in volumetric blood flow in the experimental groups, despite decreased expression of VEGF and FGF2 on 14 and 21 days. On day 21, the maximum increase in volumetric blood flow was in the pVax1-VEGF-FGF2-DsRed group. In turn, the maximum number of capillaries at 21 days was in the pVax1-VEGF-DsRed group. Capillary density was increased in pVax1-VEGF-FGF2-DsRed, and pVax1-FGF2-DsRed groups compared to control groups. We also observed low expression levels of caspase-3 and caspase-9 in the muscles of the experimental groups.
Thus, co-expression of VEGF and FGF2 stimulates angiogenic and regenerative processes in a rat model of hind limb ischemia. In addition, the results of this study are consistent with our previous work and confirm the effectiveness of using systems based on 2A-peptide sequences for transgene co-expression. The study of the serum cytokine profile showed the absence of adverse immune effects, indicating the safety of the non-viral constructs used. These results suggest the possibility of using these non-viral constructs to enhance therapeutic angiogenesis in the treatment of ischemic diseases. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

Keywords: hind limb ischemia; cytokines; angiogenesis; plasmid constructs; VEGF; FGF2; non-viral delivery

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