POSSIBLE MECHANISMS OF THE PROANGIOGENIC ACTION OF MICROPARTICLES DERIVED FROM PLATELET AND BONE MARROW

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Microparticles originate from shedding of cell membrane and are rich in membrane receptors suggesting they may play role in neovascularization *in vivo*.

The aim of the study was to elucidate mechanism of the clinical improvement considering the possible role of microparticles after the local implantation of bone marrow mononuclear cells into the critically ischemic limb.

Mononuclear cells, isolated from patient with critic limb ischemia, were enriched in AC133+ cells by employing MiniMacs magnetic beads (Miltenyi, Auburn, CA) and microparticles derived from bone marrow (BMP) were isolated. Blood platelets were collected from healthy volunteers, and activated by thrombin to obtain platelet derived microparticles (PMP). Microparticles were examined by flow cytometry analysis using anti-human antibodies against VE-Cadherin,CD41, CD62P, CD31, Glycophorin A,CD34 (Becton Dickinson) and AC133 (Milteny Biotech.). The influence of microparticles on chemotaxis and proliferation of primary endothelial cells (HUVEC) and endothelial progenitor cells (EPC) was investigated. The effect of microparticles on tubulogenesis was studied in the *in vitro* 3D Matrigel model. RT PCR was performed using SUPERSCRIPT II (Invitrogen Life Technologies) and primers for the following genes: *AC133, CD36, vWF, eNOS*.

Microparticles affect angiogenesis by influencing HUVEC migration, proliferation and differentiation. PMP transfer adhesion molecules (CD36, CD41, CD62P) to the membrane of endothelial progenitor cells and promote proliferation of these cells after 48 hours. PMP up-regulate the expression of the genes connected with EPC differentiation (*vWF* and *eNOS)*.

Proangiogenic action of microparticles present in bone marrow may be responsible for neovascularization and clinical improvement after local implantation into the critically ischemic limb.

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