VEGF IMMUNOHISTOCHEMISTRY IN SKIN WOUND HEALING

Batelja Vuletić L, Brčić L, Kapitanović S, Čačev T, Jokić M, Seiwerth S. (lbatelja@mef.hr)

Institute of Pathology, University of Zagreb Medical School; Institute Ruđer Bošković, Zagreb

Skin wound healing is a complex process involving blood clotting, inflammatory reaction, new tissue formation and remodeling. Formation of new tissue is associated and depending on angiogenesis, where the key role plays vascular endothelial growth factor (VEGF) (1). VEGF is a functional gene, member of platelet-derived growth factor (PDGF) family, whose product is glycoprotein mitogen for endothelial cells (2). In many experiments interaction between VEGF and other growth factors, chemokines and proinflammatory cytokines was studied. We analyzed VEGF presentation during reparation.

We made excision skin wound 5x5 mm at the back of adult male Wistar rats. Animals were sacrificed at 4, 6, 12, 24, 48, 72 hours and 5th and 7th day after excision. Skin tissue samples were embedded in paraffin and stained with hematoxylin-eosin and immunohistochemicaly for VEGF. Analysis was performed with light microscopy, using semi quantitative scale for positivity of immunohistochemistry. Positivity of blood vessels and capillaries of epidermis, dermis, and hypodermis was analyzed separately.

The first and highest VEGF peak was recorded after 12 hours in blood vessels and capillaries of cutis. Afterwards VEGF positivity decreased, but is still present on 7th day. Subcutis blood vessels show two peaks, one at 12 hours, and the second one at 72 hours, while capillaries in subcutis have only peak at 12 hours (Fig. 1).

Figure 1. Capillaries in subcutis at 12 hours after the injury. (VEGF immunohistochemistry, objective 25x)

We studied the mRNA expression of VEGF, isoform A. Today, real time PCR is the method of choice for quantification of mRNA transcription. VEGF has changed and we have found maximal levels at 4 hours after injury; dCtK 6.52 and second peak 48 hours after injury; dCtK 2.81. (Fig. 2)
Figure 2. Real time PCR for mRNA of VEGF

We showed that mRNA VEGF and protein VEGF in control group is in accordance with regeneration process.

References: