THE ROLE OF VEGF IN GLUCOCORTICOID INDUCED FEMORAL HEAD NECROSIS

Wolf R. Drescher1, Deike Varoga2, Rolf Mentlein3, Wolf Petersen4, Bernhard Tillmanns5, Thomas Pufe6
1Dept. of Orthopaedics, Frankfurt University Hospital, Frankfurt, Germany; 2Dept. of Traumatology, Kiel University Hospital, Kiel, Germany; 3Institute of Anatomy, Christian-Albrechts-University, Kiel, Germany; 4Dept. of Traumatology, University Hospital, Münster, Germany; 5Institute of Anatomy, Christian-Albrechts-University, Kiel, Germany; 6Institute of Anatomy, Christian-Albrechts-University, Kiel, Germany

Introduction: Femoral head necrosis (FHN) is a common complication after high dose corticosteroid treatment. In the current study we investigated the possible role of vascular endothelial growth factor (VEGF) and its receptors VEGFR-2 in steroid induced femoral head necrosis.

Materials and Methods: Femoral heads (n=6) were obtained from patients undergoing total hip arthroplasty (age 32-54) for steroid related late stage (ARCO IV) femoral head necrosis.

Human osteoblasts were derived proliferating from Oligene (Berlin, Germany) and cultured under standard conditions. Cells were incubated for 24 hours or 24 and 72 hours with dexamethasone (100 μM) and epidermal growth factor (EGF; TEBU, Offenbach, Germany), diluted to 10 ng/ml. Stimulation experiments were carried out in triplicates. Cell culture experiments were repeated for n=6 times. For histology and immunohistochemistry, tissue samples were fixed in 3% paraformaldehyde, embedded in paraffin, sectioned, dewaxed, irradiated at 750 W in a microwave oven in 0.01 M sodium citrate buffer, pH 6.0 (twice for 5 min), sections blocked with 3% hydrogen peroxide (endogenous peroxidases) and subsequently with normal serum (1:5 in Tris-buffered saline) of the species in which the primary antibody was raised, immunostained with anti-VEGF or anti-VEGFR-2 followed by biotinylated secondary antibodies and a peroxidase-labelled streptavidin-biotin staining technique; nuclei were counterstained with hemalum. AZAN, Goldner and van Giesson staining were performed according to standard protocols.

For ELISA, culture supernatants and bone samples of necrotic femoral heads were analysed by a sandwich ELISA (R&D Systems, Minneapolis, MN, USA) that detects all VEGF splice forms. Human recombinant VEGF165 (PreproTech, Rocky Hill, NJ, USA) served as standard.

Results: Histology confirmed later stage femoral head necrosis (ARCO IV), and deformity of the femoral head. Immunohistochemistry was performed to evaluate the expression of the bone remodeling factor VEGF in case of necrotic bone disease of the femur.

Within all sections of necrotic femoral heads VEGF could be immunostained within the intra- and pericellular matrix of osteoblasts in the necrotic bone area. VEGF immunostaining could be abolished by pre-incubation of the primary antibody with recombinant VEGF or by omitting the primary antibody. Osteoblasts of the non-necrotic bone area were clearly less immunoreactive to VEGF antibody. Osteoblasts of necrotic bone areas could also be immunostained for the VEGF receptor-2 (KDR). According to the staining intensity, VEGF and KDR-expression was clearly upregulated in case of steroid related femoral head necrosis in necrotic bone areas. ELISA confirmed VEGF induction in necrotic bone areas of femoral heads (Figure 1). Compared to non-necrotic bone samples, VEGF expression increases from 100pg/ml to 490 pg/ml .

To test whether cultured primary osteoblasts express VEGF and its receptors and if they were suitable for stimulation experiments, RT-PCR examinations were performed. Osteoblasts express the splice variants VEGF121 and VEGF165. We also analyzed the VEGF-receptor expression by RT PCR experiments for verification of the immunohistochemical data. We detected one band corresponding to the VEGFR-2 (555 kb).

To evaluate the influence of dexamethasone on VEGF expression in osteoblasts, stimulation experiments were performed. ELISA experiments demonstrated, that osteoblast-derived VEGF production was stable under culture conditions for at least 72 hours. VEGF amounts in culture supernatants increased 2.9 – fold (from 164.45 +/- 24 pg/ml to 476.5 +/- 64.85 pg/ml). After co-incubation with 100 μM dexamethasone for 24 hours, VEGF expression levels were significantly (p ≤ 0.001) decreased in the collected cell supernatants (control: 164.45 pg/ml; dexamethasone: 64.2 +/- 6.95 pg/ml). Addition of the growth factor EGF (10ng/ml), which has a stimulatory influence on VEGF expression in different cells, failed to induce osteoblast-derived VEGF expression in presence or absence of glucocorticoids.

Discussion: The current study demonstrates an increased expression of VEGF in osteoblasts from necrotic femoral heads and this may be connected with the process of revascularization and bone ongrowth into the necrotic area as the femoral heads were harvested in later stage osteonecrosis. Ohzono et al. described that reparative arterioles grew on the trunk of the intracapital nutrient arteries (1). They also described an avascular zone directly subchondrally, an underlying reparative vascular zone, and a normal vascular zone. In the reparative vascular zone, they described an advancing ingrowth of reparative vessels from stage 2 to 4. These were described as sparse in stage 2, a fine network in stage 3, and a dense network in stage 4. The new results of increased VEGF expression in the necrotic femoral heads of the present study well correlate with the findings of Ohzono et al. Vascular endothelial growth factor is increased, and stimulates the ingrowth of reparative vessels into the necrotic femoral head.

On the other hand, the decrease in VEGF detected in the in-vitro part of the present study in osteoblasts incubated with GC may support the development of initial osteonecrosis. This may be an additional pathogenic factor in the early stage of femoral head necrosis. GC impairs vessel ingrowth into the endarterial bed of the femoral head (18), and makes it more vulnerable to ischemia. Vessels have been shown to be essential to the process of revascularization and bone ongrowth into the necrotic area as the femoral heads were harvested in later stage osteonecrosis. Ohzono et al. described that reparative arterioles grew on the trunk of the intracapital nutrient arteries (1). They also described an avascular zone directly subchondrally, an underlying reparative vascular zone, and a normal vascular zone. In the reparative vascular zone, they described an advancing ingrowth of reparative vessels from stage 2 to 4. These were described as sparse in stage 2, a fine network in stage 3, and a dense network in stage 4. The new results of increased VEGF expression in the necrotic femoral heads of the present study well correlate with the findings of Ohzono et al. Vascular endothelial growth factor is increased, and stimulates the ingrowth of reparative vessels into the necrotic femoral head.


Acknowledgements: The study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft) grants no. 449/ DR 2-1, VA 220/2-1 and PU214/4-2 and PU214/3-2.

Fig. 1. VEGF expression was clearly upregulated in the necrotic area of human femoral heads.