THE ROLE OF VEGF (VASCULAR ENDOTHELIAL GROWTH FACTOR) IN GLUCOCORTICOID INDUCED OSTEOPOROSIS IN VITRO AND IN VIVO

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INTRODUCTION:
Osteoporosis is a disease characterized by low bone mass and an increased susceptibility to fractures. The molecular mechanism of osteoporosis and the occurrence and function of related cytokines are largely unclear. The role of vascular endothelial growth factor (VEGF), an angiogenic protein and a chemoattractant for macrophages, in Osteoporosis is not investigated so far. We show in a Göttinger Minipig model that VEGF is highly expressed in osteoblasts of vertebra and decreased under glucocorticoid (GC) influence. The aim of the study was to investigate the influence of GC treatment on the VEGF expression.

MATERIALS AND METHODS:
As part of a larger study, 20 primiparous sows were allocated to 2 experimental groups when they were 15 months old: Control group and GC treatment for 15 month. Animals were fed a semisynthetic diet until they were sacrificed. Group GC received prednisolon at a daily dose of 1 mg/kg body weight for 8 weeks and thereafter 0.5 mg/kg body weight. The 8th thoracic vertebra was investigated.

For immunohistochemistry, tissue samples were fixed in 3% paraformaldehyde, embedded in paraffin, immunostained with anti-VEGF (1:40 in Tris-buffered saline, 60 min; sc7269 mouse monoclonal IgG2a, Santa Cruz Biotechnology, CA, USA) or anti-VEGFR-2 (1:40; sc-6251 monoclonal IgG1, Santa Cruz Biotechnology).

Enzyme-linked immunosorbent assay (ELISA): ELISA (R&D Systems, Minneapolis, MN, USA) that detects all VEGF splice forms. Human recombinant VEGF (PreproTech, Rocky Hill, NJ, USA) served as standard.

Reverse transcription-polymerase chain reaction (RT-PCR) for VEGF. For RT-PCR the following primers / conditions were applied: VEGF, pVEGFas 5’-ATG CGA TCC ACA AAA CCT CAC C-3’ (sense) and pVEGFas 5’-ATG TGG TTC CCG AA CGC TG-3’ (antisense) with 40 cycles at 60°C annealing temperature yielding a 303 product; pbeta actin 5’-CTT CCT GGG CAT GGA ATC CT-3’ (sense) and pbeta actin 5’-GAT CTT GAT CTT GCT GCT-3’ (antisense) yielding a 193 bp product.

Culture and stimulation of human Chondrocytes and osteoblasts: Human Chondrocytes were obtained from Mary Goldring (Harvard, Boston, USA); human Osteoblasts from PromoCell (Heidelberg, Germany). For stimulation the medium was replaced by DMEM (without FCS), and the cells exposed to the stimulators for 24 h. Conditioned medium was withdrawn, and aliquots assayed for VEGF content. The cells were washed with phosphate-buffered saline, lysed, the DNA content was measured fluorometrically with the CyQuant reagent (Molecular Probes, Eugene, Oregon, USA), and related to a standard number of cells (counted with trypsinised cells). Human recombinant EGF (PreproTech), Dexamethasone (Sigma-Aldrich) and Hydrocortison (PromoCell, Heidelberg, Germany) was used for stimulation.

Results:

VEGF concentrations are strongly decreased in the thoracic vertebra of GC treated Minipigs. Samples from the 8th thoracic vertebra were homogenized in buffer, and immunoreactive VEGF determined in the homogenates by an ELISA detecting all VEGF splice variants. n= 10 different sows and n = 10 controls.

Fig. 1:

VEGF can be immunostained in osteoblasts and cells of the endost. Bar = 100 μm; original magnifications 45- fold. The VEGF receptor-2 can be detected by immunohistochemistry on osteoclasts. Bar = 10 μm; original magnifications 600-fold.

Fig. 2:

These data strongly suggest that VEGF plays an important autocrine or paracrine role in the progression of osteoporosis. Since VEGF is a potent angiogenic peptide, VEGF should be responsible for the neovascularisation observed in remodeled bone. There is no correlation between VEGF amount and osteoclast activation and related bone loss like postulated by Kaku et al. (2001) in a different model. The cause for the decreased VEGF levels in steroid induced osteoporosis might be the antiinflammatory effect of steroid treatment. Beyond it the GC can act directly on the osteoblast because osteoblast also have GC-receptors (Dovio et al. 2001). Low VEGF levels in this animal model of steroid induced osteoporosis might be responsible for the little bone assembly. So it seems that VEGF is a bone formation promoting factor and not participated in trabecular bone reduction.

Influence of GC and growth factors on VEGF secretion from cultivated osteoblasts and chondrocytes. Conditioned media from osteoblasts and chondrocytes incubated for 24 h with or without epidermal growth factor (EGF, 10 ng/ml), Dexamethasone or Hydrocortison (100 nM) without foetal calf serum were analysed for VEGF concentration by ELISA (mean +/- standard deviations from n = 3 cultures each). VEGF concentrations in the culture supernatants are moderately increased by stimulation with EGF alone, but decrease strongly after combined application with dexamethasone or hydrocortison.

Discussion: These data strongly suggest that VEGF plays an important autocrine or paracrine role in the progression of osteoporosis. Since VEGF is a potent angiogenic peptide, VEGF should be responsible for the neovascularisation observed in remodeled bone. There is no correlation between VEGF amount and osteoclast activation and related bone loss like postulated by Kaku et al. (2001) in a different model. The cause for the decreased VEGF levels in steroid induced osteoporosis might be the antiinflammatory effect of steroid treatment. Beyond it the GC can act directly on the osteoblast because osteoblast also have GC-receptors (Dovio et al. 2001). Low VEGF levels in this animal model of steroid induced osteoporosis might be responsible for the little bone assembly. So it seems that VEGF is a bone formation promoting factor and not participated in trabecular bone reduction.

The crucial role of angiogenesis factors like VEGF is supported by the coupling model of Parfitt (2000). He describes the coupling of osteoblasts and osteoclasts as so called BMUs (basic multicellular units) orchestrated by endothelial cells of a capillary in the heart of a BMU. The angiogenesis factor (VEGF) recruits the endothelial cells and the endothelial cells themselves organize the bone remodeling by time dependent gene switch. If the endothelial cells are once recruited, they could also release a potent mitogen for osteoblasts (Guenther et al. 1986). Beyond it they could also inhibit and regulate osteoclast activity (Zaidi et al. 1993).