The role of vascular endothelial growth factor in glucocorticoid-induced bone loss: evaluation in a minipig model

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Abstract

Vascular endothelial growth factor (VEGF) has been recently shown to play an important role during endochondral bone formation in hypertrophic cartilage remodeling, ossification, and angiogenesis. To our knowledge there are no previous studies investigating the role of VEGF in osteoporosis. We hypothesized that VEGF expression in bone would be reduced under glucocorticoid (GC) treatment and tested this in a minipig model. As part of a larger study, 17 primiparous sows (Göttingen minipig) were allocated to two experimental groups when they were 15 months old: a control group (n = 9) and a group receiving GC treatment for 15 months (n = 8). All animals were fed a semisynthetic diet until they were sacrificed. The GC group received prednisolone orally at a daily dose of 1 mg/kg body wt for 8 weeks and thereafter 0.5 mg/kg body wt. VEGF levels in lumbar vertebrae were measured by enzyme-linked immunosorbent assay (ELISA) and verified by Western blot analysis. VEGF and its receptors (VEGFR) were localized by immunohistochemistry. Expression of VEGF-mRNA was analyzed by reverse transcription-polymerase chain reaction. VEGF protein was quantified in supernatants of cultivated osteoblasts by ELISA. Spinal bone mineral density was assessed in vivo by quantitative computed tomography. Expression of cyclooxygenase-2 (COX-2) protein was investigated by immunohistochemistry. High VEGF concentrations were measured in normal lumbar vertebrae whereas VEGF concentrations were 60% lower (P < 0.0001) in GC-treated minipigs. VEGF levels were associated (r = 0.7) with rates of spinal trabecular bone loss, which differed significantly (P < 0.001) between controls (0.47 ± 2.2% SEM) and GC-treated minipigs (12.8 ± 2.3% SEM). Osteoblasts were immunopositive for VEGF. VEGF receptors VEGFR-2 (KDR, flk-1) and VEGFR-1 (flt-1) could be immunostained on osteoclasts and osteoblasts. VEGF-mRNA and protein were detectable in the lumbar vertebrae of all animals. The expression of COX-2 protein was decreased in GC-treated animals. VEGF is produced in osteoblasts and its concentration is decreased in GC-treated animals as well as in osteoblasts exposed to GC. Since reductions in VEGF concentrations correlate with parallel measurement of bone mineral density in GC-treated minipigs we hypothesize that VEGF may be an important modulating factor for bone remodeling, specifically in GC-induced osteoporosis. GC inhibit COX-2 and hence prostaglandin E2 (PGE2) production. Since PGE2 is able to increase VEGF synthesis, this may be the link between GC and VEGF decrease.

Keywords: Glucocorticoids; Bone mineral density; QCT; VEGF; VEGFR; VEGF-mRNA; Bone; Osteoblast culture; Minipig

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Introduction

Osteoporosis is characterized by low bone mass and an increased susceptibility to fractures. Many factors affect bone mineral density including menopause, age, activity, diet, or drugs. For example, osteoporosis is an important side effect of long-term treatment with glucocorticoids (GC) [1]. However, the molecular mechanism of GC-induced osteoporosis is complex and the occurrence and function of related cytokines is the subject of ongoing research.

Osteoporosis is characterized by an imbalance between bone resorption and bone formation. In bone multicellular units (BMUs, [2]) new osteoblasts appear only at sites recently vacated by osteoclasts, a phenomenon referred to as coupling [3]. Despite the importance of this process for various bone diseases its regulation is not completely understood; various growth factors such as transforming growth factor (TGF-β [4] or insulin-like growth factor (IGF-1), biomechanical factors [5], or changes in the cytokine network of the bone marrow [6] have been proposed. The parathyroid hormone-related protein (PTHrP) also seems to be involved in modulation of endochondral bone formation [7,8]. Although most of these mechanisms might contribute to coupling, direct evidence is still lacking [3]. For example, increase in mechanical strain can only be responsible in specific regions of the skeleton.

Blood vessels are present in each BMU. The location of vessels in the center of the BMU between the osteoclasts advancing at the end of the cavity and the osteoblasts lining the walls farther behind is strategically perfect for participation in coupling. Since the capillaries in bone grow at the same rate as the BMU advances [9], angiogenesis—the formation of new vessels from preexisting capillaries—might be an integral process during bone remodeling and the sequential expression of peptides during angiogenesis might have a strong impact on the mechanism of coupling.

One of the most important angiogenic peptides, vascular endothelial growth factor (VEGF, also termed vascular permeability factor), has been recently identified as the essential factor for endochondral ossification, a necessary process for growth of long bones whereby cartilage is replaced by bone [10]. The invasion of blood vessels into cartilage, which is normally avascular, is the crucial first step in this process, and the vasculature provides a conduit for the recruitment of the cell types involved in cartilage resorption and bone deposition. During normal bone morphogenesis, chondrocytes in the epiphyseal plate become hypertrophic, produce angiogenic factors, and finally undergo apoptosis. VEGF expression occurs in the same cell population that is invaded by new vessels (hypertrophic chondrocytes; [10]). Furthermore, apoptosis of terminal hypertrophic chondrocytes is delayed and recruitment of chondroclasts, monocyte-derived cells responsible for cartilage resorption, is reduced after inhibition of VEGF action by binding the protein to a soluble receptor [11]. According to the model of vascular coupling proposed by Parfitt [3], we postulated that angiogenesis factors are important in bone remodeling and should be affected by drugs known to impair bone formation, such as glucocorticoids. VEGF is a homodimeric, heavily glycosylated protein of 46-48 kDa. The two subunits of about 24 kDa are linked by disulfide bridges. There are several isoforms of human VEGF of 121, 145, 165, 189, or 206 amino acids, arising by alternative splicing of a single mRNA. These isoforms differ in their molecular masses and in their biological properties such as their ability to bind to heparin or heparan-sulfate proteoglycans and to different VEGF receptors (VEGFR). The splice forms VEGF121, VEGF145, and VEGF165 are secreted, whereas VEGF189 is tightly bound to cell surface heparan-sulfate and VEGF206 is an integral membrane protein [11]. In contrast to the other forms, VEGF121 does not bind to heparin or extracellular matrix proteoglycans. The signaling tyrosine kinase receptors VEGFR-1 (fit-1, fms-like tyrosine kinase-1) bind VEGF121 and VEGF165, while VEGFR-2 (KDR, kinase domain region/flk-1, fetal liver kinase-1) additionally binds VEGF145 (apart from certain VEGF-related peptides). The coreceptors neuropilin-1 and -2 bind selectively the 165-residue VEGF isoform, which is the most common variant in the majority of tissues [11].

The role of VEGF in osteoporosis has not been investigated so far. Harada et al. [12,13] detected an expression of VEGF in human osteoblasts and they showed that prostaglandin E2 (PGE2) increased VEGF mRNA levels. Martinez et al. [7] could show an age-related decrease in the secreted levels of VEGF in osteoblasts. Further, they observed an increase of VEGF and, respectively, a decrease of PTHrP after 1,25(OH)2 vitamin-D3 incubation. We investigated the possible role of VEGF and its receptors VEGFR-1/-2 in steroid-induced bone loss and bone remodeling by biochemical and immunohistochemical methods. Using the Göttingen minipig as a model, our aim was to investigate associations of VEGF levels with GC treatment and the bone loss induced.

Materials and methods

Animal experiment

As part of a larger study [14], seventeen 29.6 ± 11.1 month old female, primiparous Göttingen minipigs of the own breeding herd of the Federal Dairy Research Centre,
Kiel, were used as a large animal model for glucocorticoid-induced osteoporosis. The animals were skeletally mature as radiographically evidenced by closure of the growth plate.

Sows were allocated to two experimental groups, a control group (n = 9) and a GC treatment group (n = 8), and were fed a semisynthetic diet which provided all nutrients in a sufficient amount required for adult animals. The feed supply was restricted to avoid fattening and was based on the metabolic body weight. The steroid-treated group received prednisolone orally at a daily dose of 1 mg/kg body wt for 8 weeks and thereafter 0.5 mg/kg body wt for 56 weeks. Bone mineral density was assessed in vivo. After a study duration of 15 months the animals were sacrificed. The fifth lumbar vertebrae was taken and frozen at −18°C until analysis.

Approval to perform the animal experiment was obtained from the Ministerium für Natur, Umwelt, und Landesentwicklung des Landes Schleswig-Holstein.

Tissues

Immunohistochemistry

For immunohistochemistry, tissue samples were fixed in 3% paraformaldehyde, embedded in paraffin, sectioned, dewaxed, and irradiated at 750 W in a microwave oven in 0.01 M sodium citrate buffer, pH 6.0 (twice for 5 min) and sections were 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 (1:40 in Tris-buffered saline, 60 min; sc7269 mouse monoclonal IgG2a, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-VEGFR-2 (1:40; sc-6251 monoclonal IgG1, Santa Cruz Biotechnology) or anti-VEGFR-1 (1:40; sc-316 affinity-purified polyclonal antibody, Santa Cruz Biotechnology) or anti COX-2 (1:20; sc-7951 rabbit polyclonal antibody, Santa Cruz Biotechnology), followed by biotinylated secondary antibodies and a peroxidase-labeled streptavidin–biotin staining technique; nuclei were counterstained with hemalum.

Enzyme-linked immunosorbent assay (ELISA) and Western blot

For ELISA, frozen tissue samples were crushed in an achate mortar under liquid nitrogen and homogenized in 150 mM NaCl 20 mM Tris/HCl buffer, pH 7.4, a soluble fraction was obtained by centrifugation (48,000g, 60 min), and aliquots (100 µl) were analyzed by a sandwich ELISA (R&D Systems, Minneapolis, MN, USA) that detects all VEGF splice forms. Human recombinant VEGF65 (PreproTech, Rocky Hill, NJ, USA) served as standard. For Western blots, samples were reduced in the presence of 10 mM dithiothreitol, proteins were separated by sodium dodecyl-sulfate–polyacrylamide gel electrophoresis (10% gels) and transferred onto nitrocellulose membranes that were blocked and incubated with antibodies according to standard techniques as described [15]. Signals were detected by chemoluminescence reaction (ECL-Plus; Amersham–Phar- macia, Uppsala, Sweden) in a Fuji LAS 1000 Imager.

Reverse transcription-polymerase chain reaction (RT-PCR) for VEGF

For RT-PCR, frozen samples (100 mg) were crushed in an achate mortar under liquid nitrogen and homogenized in 5 ml “peggold RNA Puer” solution (peqLab Biotechnologie, Erlangen, Germany) with a Polytron homogenizer, insoluble material was removed by centrifugation (12,000g, 5 min, 4°C), and RNA was isolated as described by the manufacturer (phenol–guanidinium thiocyanate method). Crude RNA was purified by isopropanol and repeated ethanol precipitation, and contaminating DNA was destroyed by digestion with RNase-free DNase I (20 min 25°C; Boehringer, Mannheim, Germany). After inactivation the DNase (15 min 65°C) cDNA was generated with 1 µl (20 pmol) oligo (dT)15 primer (Amersham–Pharmacia Biotech, Uppsala, Sweden) and 0.8 µl superscript RNase H− reverse transcriptase (Gibco, Paisley, UK) for 60 min at 37°C. For PCR, 4 µl cDNA was incubated with 30.5 µl water, 4 µl 25 mM MgCl2, 1 µl dNTP, 5 µl 10× PCR buffer, and 0.5 µl (2.5 U) Platinum Taq DNA polymerase (Gibco) and 2.5 µl (10 pmol) of each primer pair. The following primers/ conditions were applied: VEGF, pVEGFs 5′-ATG CGG ATC AAA CCT CAC C-3′ (sense) and pVEGFas 5′-ATC TGG TTC CCG AA CGC TG-3′ (antisense), with 40 cycles performed at 60°C annealing temperature, yielding a 303-bp product; β actin s 5′-CTT CCT GGG CAT GGA ATC CT-3′ (sense) β actin as 5′-GAT CTT CAT CCT GAT CGT GCT-3′ (antisense), yielding a 193-bp product.

Bone densitometry

Trabecular bone mineral density (BMD) of the third lumbar vertebral body was assessed in vivo by quantitative computed tomography (QCT) at 15 months. Data were acquired on a Siemens Somatom Plus scanner and calibrated using the Siemens BMD calibration phantom. An ellipsoid region of purely trabecular bone was evaluated in the central 8-mm-thick slice in the center of the vertebrae.

Statistical analysis

Differences between the GC and control group were evaluated using the t test. Group differences were considered significant if P < 0.05. All statistical analyses were
carried out using the JMP statistics package (SAS Institute, Cary, NC, USA).

**Results**

**VEGF and VEGFR-1 and -2 could be immunostained in osteoblasts of lumbar vertebrae**

Osteoclasts were immunopositive for VEGFR-1 and -2

Within all sections of lumbar vertebrae from control and GC-treated animals, VEGF could be immunostained within the intra- and pericellular matrix of osteoblasts (Fig. 1). The VEGF immunostaining could be abolished by preincubation of the primary antibody with recombinant human VEGF or by omitting the primary antibody (not shown).

Fig. 1. VEGF can be immunostained in osteoblasts. Immunostaining is abolished by preincubation of the primary antibody with recombinant human VEGF or after omission of the primary antibody. Bar = 10 μm; original magnification 600-fold.

Osteoclasts (Fig. 2; arrowheads) and osteoblasts (Fig. 2; arrows) could be immunostained for the VEGF receptors VEGFR-2 (KDR) and VEGFR 1 (FLT-1).

A decrease of VEGF could be measured by ELISA in lumbar vertebrae after GC treatment

The removed fifth lumbar vertebrae of the control group showed concentrations of 216 ± 13 pg/100 mg fresh tissue (mean ± standard deviation) as measured by ELISA, whereas in corresponding samples from GC-treated animals VEGF strongly decreased by about 60% 86 ± 14 pg/100 mg fresh tissue (P ≤ 0.001, Fig. 3).

Fig. 2. The VEGF receptor-1 (VEGFR-1, also termed flt-1) and -2 (VEGFR-2, also termed KDR or flk-1) can be detected by immunohistochemistry on osteoclasts (arrowheads) and osteoblasts (arrows). Immunostaining is abolished by preincubation of the primary antibody with recombinant human VEGF-R or after omission of the primary antibody. Bar = 10 μm; original magnification 600-fold.

Fig. 3. VEGF concentrations are strongly decreased in the lumbar vertebrae of glucocorticoid-treated minipigs. Samples from the fifth lumbar vertebra of GC-treated minipigs and of controls were homogenized in buffer, and immunoreactive VEGF was determined in the homogenates by an ELISA detecting all VEGF splice variants. n = 8 for GC-treated sows and n = 9 for controls.

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VEGF mRNA and protein could be detected in lumbar vertebrae

Expression of VEGF-mRNA by RT-PCR with primers yielding a 303-bp product is shown in Fig. 4. VEGF-mRNA PCR products were obtained from all samples of the investigated lumbar vertebrae. We also analyzed the VEGF protein level by Western blot experiments for verification of the complete expression. One band corresponding to the dimer of the splice variant VEGF 165 is detectable (Fig. 4).

Our results demonstrate that minipigs suffer from bone loss induced by GC paralleling the reduction in VEGF concentrations

BMD assessed by QCT at start of the experiment when the sows were 29.6 ± 11.1 months old was comparable for the control group and the GC-treated group. Over the 15-month duration of the study BMD was stable in the control group \( (n = 9) \) (\(-0.46 \pm 2.2\% \) standard error of the mean (SEM), n.s., SD = ±7.4\%), but decreased in the GC group \( (n = 8) \) by 58.4 ± 11.1 mg/cm\(^3\) SEM \( (P < 0.001) \), SD = 26.1 mg/cm\(^3\), or -12.8 ± 2.3\% SEM, SD = 5.5\% (Fig. 5). The difference in the rates of loss was highly significant \( (P < 0.0013) \). VEGF levels were significantly correlated with this change in BMD \( (r = 0.7) \).

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Fig. 4. VEGF protein can be detected by Western blot (WB, 45 kDa; lane 1, control; lanes 2 and 3, GC treated) and VEGF mRNA by RT-PCR in all lumbar vertebrae (only three are shown) yielding a 303-bp product. A separate RT-PCR for \( \beta \)-actin yielding a 193-bp product served as control for intactness of the mRNA applied and for equal amounts; control (lanes 1 and 3), GC treated (lane 2).

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COX-2 protein is strongly decreased in GC-treated animals

COX-2 can be immunostained in osteoblasts and adipocytes of untreated animals. COX-2 protein is strongly decreased in osteoblasts and adipocytes of the lumbar vertebrae of glucocorticoid-treated animals (Fig. 6).

Discussion

Coupling of resorption by osteoclasts and bone formation by osteoblasts results in ongoing turnover of bone mass [2]. There are several indications that vasculature plays an important role for the mechanism of coupling [3,16]. Between bone endothelium and osteoblasts seems to be an important role for the mechanism of coupling [3,16]. Between bone endothelium and osteoblasts seems to be a functional relationship with an unclear mechanism [17]. Recent reports document the important role of angiogenic factors in skeletal development and in other disorders of the skeleton [10,18,19]. For example, the first step of enchondral ossification is the expression of VEGF by hypertrophic chondrocytes [10,18,20]. Under pathologic conditions VEGF is strongly expressed during the time course of fracture healing [19]. During the remodeling of the osseous fracture callus VEGF is expressed by osteoblasts while the receptors of VEGF, VEGFR-1 and VEGFR-2, are expressed by osteoclasts [19]. Thus, it appeared promising to investigate the role of angiogenic factors, specifically VEGF, in osteoporosis.

In our study of GC-induced bone loss in minipigs we indeed observed strong significant differences in the levels of VEGF expression in lumbar vertebrae: the VEGF level in minipigs on GC treatment was 57% lower compared to the corresponding level in control animals ($P < 0.0001$). In fact, there was no overlap between the ranges of VEGF of the control and the GC group, with a dividing line around 150 pg/ml (Fig. 3).

For the pig or minipig model only a few studies have been published on cartilage or bone metabolism with attention to bone loss or microstructural changes [21–29]. To our knowledge, there are no published data on mature minipigs as a model for steroid-induced bone loss. The results reported here have been obtained on animals that are part of a larger project. In that study we previously reported significant bone loss in GC-treated animals [14,29]. In the subgroup investigated here we observed that BMD at the end of the treatment period was 13% lower in GC-treated animals. VEGF levels were significantly correlated with this change in BMD ($r = 0.7$). Although not sufficient to demonstrate a causal relationship, the substantial intergroup difference is compatible with our hypothesis that GC-induced impairment of angiogenesis may contribute to GC-induced bone loss. The correlation between low VEGF amount and high rates of bone loss suggests that VEGF is not only an osteoclast activator as postulated by Kaku et al. [30] in a different model. Our results are consistent with the hypothesis of Parfitt [3]. Nevertheless, we cannot exclude the idea that other cytokines like IGF-1 may also be involved in the GC effect. Osteoblasts and osteoclasts were immunopositive for the VEGFR-1 and -2. RT-PCR could verify these findings on the mRNA level and Western blot experiments on the protein level. The expression of VEGF in normal bone might indicate that VEGF plays a physiologic role in bone turnover. Crosstalk between osteoblasts and adjacent endothelial cells in view of altering the vascularity of the bone forming site is also possible. It has been previously described that the expression of VEGF can be enhanced by several growth factors like PGE2, FGF-2, TGF-β1, IGF-1, and BMP-4, -2, and -6 [14,31–36]. bFGF stimulates VEGF release through p42/p44 MAP kinase in osteoblasts and negatively regulates by p38 MAP kinase [37]. Harada et al. [12,13] showed that PGE2 increased VEGF mRNA levels in osteoblastic RCT-3 cells and in rat calvaria-derived osteoblast-enriched cells. Migita et al. [38] examined the down-regulation of COX-2 in response to glucocorticoids in human rheumatoid synovial fibroblasts. PGE2 is synthesized by COX-2 [38]. By immunohistochemistry we could show that COX-2 expression is reduced in GC-treated animals. Taken together, GC could reduce VEGF levels by down-regulating COX-2 with subsequent reduction of PGE2.

Apart from these various cytokines VEGF expression is also influenced by physical and mechanical factors. VEGF expression in osteoblasts is modulated by the extracellular microenvironment like acidic pH, which significantly decreases VEGF production [39]. Exposure of osteoblasts to hypoxia induces VEGF expression via induction of the transcription factor hypoxia-inducible factor (Hif-2-α) and transcriptional activation of the VEGF promoter [40,41]. Zheung et al. [42] showed that VEGF expression in cells is sensitive to mechanical stimuli. The application of intermittent cyclic stretching increased VEGF expression in cardiac fibroblast [42]. It would be interesting to further investigate the role of VEGF in mechanotransduction.

By a range of different methodological approaches we have demonstrated that VEGF and its receptors, VEGFR-1 and -2, are expressed by endothelial cells, osteoblasts, and osteoclasts. These data strongly suggest that VEGF plays an important autocrine or paracrine role in bone remodeling and potentially in the progression of osteoporosis. Since VEGF is a potent angiogenic peptide, it is likely to be responsible for the neovascularization observed in remodeled bone. The crucial role of angiogenesis factors like VEGF is supported by the coupling model of Parfitt [3]. He describes the coupling of osteoblasts and osteoclasts in the basic multicellular units as 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 a time-dependent gene switch. Once endothelial cells are recruited, they could also release a potent mitogen for osteo-
busts [43]. Beyond this they could also inhibit and regulate osteoclast activity [44].

In conclusion, we have demonstrated a strong GC-associated reduction in VEGF expression in Göttingen minipigs which correlates with the GC-associated bone loss. The correlation between decreased VEGF levels and bone loss supports our hypothesis that VEGF-modulated vascularization of the BMU may represent an important aspect of bone turnover, specifically in GC-induced bone loss. Future studies should elucidate the role of VEGF in primary osteoporosis. Whether its upregulation could have a stimulating therapeutic effect on bone formation remains to be investigated.

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References


