

Expression of Vascular Endothelial Growth Factor on Chondrocytes Increases with Osteoarthritis - An Animal Experimental Investigation

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Abstract: *Objective:* To evaluate the expression of VEGF by chondrocytes of hyaline cartilage during the course of osteoarthritis (OA).

Methods: In 12 white New Zealand rabbits the anterior cruciate ligament (ACL) was resected to create an anterior instability of the knee. In 12 control rabbits only a sham operation without resection of the ACL was done. Four animals of each group were killed at 3, 6, and 12 weeks. The load bearing area was evaluated histologically according to Mankin and by immunostaining for VEGF.

Results: In the experimental group, histological grades of OA showed a positive linear correlation with the time after surgery. Immunostaining showed an increased expression of VEGF in the control group after 3 weeks, which dropped to normal after 6 weeks. There was no difference in the progression of OA between control and experimental groups after 3 weeks, but a significant difference was seen after 6 ($p=0,01$) and 12 ($p=0,05$) weeks. A significant positive correlation between VEGF expression and the histological grade of OA was found ($r = 0.767$; $p<0.01$).

Conclusions: An increase of VEGF expressing chondrocytes occurs during time course of OA.

Keywords: VEGF, animal experiments, osteoarthritis.

INTRODUCTION

Osteoarthritis is a degenerative disease of the hyaline cartilage of the articular surfaces, directly related to ageing and mechanical damage [1], resulting in matrix degradation [2, 3]. Several studies of late stage arthritis showed that VEGF is expressed in osteoarthritis [4-6], indicating that angiogenesis might be involved in the pathology of OA. In fact, in severe arthritis [7], angiogenesis has been observed. Little is known about the time depended expression of VEGF during the time course of OA. This study was designed to evaluate (a) the time depending expression of VEGF during the course of OA and (b) possible correlations with histological findings.

MATERIAL AND METHODS

Animals, Surgery and Macroscopic Evaluation

The experiments were performed with permission of the local government (G49/2000) in accordance with National Institute of Health (NIH) guidelines as described earlier [8]. Briefly, twenty eight fully grown, female white New Zealand rabbits were used. In the experimental group (12 animals), the anterior cruciate ligament was resected to create instability of the knee. In the control group (12 animals),

only a sham operation without resection of the ACL was done. Four animals of each group were killed at 3, 6, and 12 weeks postoperatively. In addition, four animals (0 weeks) did not undergo any treatment and were killed at the time of surgery. After slaying the animals, both distal femurs were prepared and immediately stored at -80°C . Macroscopic grading of arthritis was done as described previously [8].

Histological Evaluation

Sample preparation and immunostaining were performed as described earlier [8], with some modifications. Briefly, frozen tissue samples were thawed, fixed with 4% paraformaldehyde, decalcified using buffered EDTA (20%, pH 7.4), dehydrated and embedded in paraffin. Sections (5 μm thick) were cut, mounted on poly-L-lysine coated glass slides, deparaffinized in xylene, and washed. Epitope unmasking was done using proteinase K digestion (DAKO, Germany) for 5 minutes and endogenous peroxidase activity was quenched by treating tissue sections with 3% H_2O_2 for 10 minutes, non-specific binding was blocked using 3% bovine serum albumin (BSA/TrisHCl), each followed by an excessive washing procedure. Sections thus prepared were incubated with monoclonal mouse IgG antibodies against VEGF diluted 1:50 in Tris/BSA (Calbiochem Clone JH 121) at 4°C over night. Visualization was done using EnVision+ System- HRP with AEC-chromogene (DAKO Envision Systems, Germany, K4001). As control instead of primary antibody, mouse IgG1 with irrelevant specificity (*Aspergillus niger* glucose oxidase,

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Fig. (6). 12 weeks after acl resection showing pronounced osteophytes and exposure of the subchondral cartilage zone.

Focusing on the time course of the control group, three weeks after surgery the percentage of VEGF expressing chondrocytes increases although no mechanic instability was induced by ACL resection. This might be due to angiogenic process common in wound healing [13, 14]. Nevertheless, it is remarkable that the histological grading according to Mankin is raised after 3 weeks and drops to normal in the following time, comparable to the percentage of VEGF expressing chondrocytes. This might be interpreted that VEGF alone has a negative impact on the integrity of the articular cartilage as postulated by Pufe [15]. This finding has far reaching consequences; for antiangiogenic therapy might be expedient in the treatment of osteoarthritis. Further studies are needed to evaluate VEGF as a target in osteoarthritis treatment.

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ABBREVIATIONS

ACL = Anterior cruciate ligament
 OA = Osteoarthritis
 VEGF = Vascular endothelial growth factor

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