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Injection of vascular endothelial growth factor into knee joints induces osteoarthritis in mice

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SUMMARY

Osteoarthritis (OA) is a common joint disorder affecting *circa* 2% of the population.
Objectives: It has been suggested that secretion of vascular endothelial growth factor (VEGF) could play a role in the chain of events leading to OA.
Methods: In the present study, healthy mice were injected intra-articularly with VEGF.
Results: Shortly after the administration of VEGF, synovial hyperplasia, increased calcification of the articular cartilage and bone sclerosis were observed. Consequently, cartilage degradation characteristic of OA was found. These changes were seen to a lesser degree in the opposite knees of VEGF-injected mice and did not occur in the control mice.
Conclusions: The findings suggest an active role of VEGF in the pathogenesis of OA and render support to a possible role for subchondral bone sclerosis in the pathogenesis of cartilage degradation.

Introduction

Osteoarthritis (OA) is a prevalent, slowly progressing joint disorder clinically manifested by pain and disability. OA is characterized by synovitis, cartilage degeneration, subchondral bone sclerosis, and osteophyte formation. Articular cartilage degradation is a hallmark of OA. It should be pointed out that changes in the subchondral bone play a major role in the onset and progression of the disease[1–3]. Initiation of OA has been correlated with either excessive or alternatively, insufficient blood flow to the subchondral bone[4]. The latter may be associated with compromised delivery of nutrients and gas exchange with the articular cartilage[5].

In OA, osteoclastic resorption of the subchondral bone is followed by reduction of structural support to the articular cartilage[6,7]. Sclerosis associated contour changes result with a decrease in the mechanical, shock-absorbing support produced by the cartilage[1]. The damage to the articular cartilage is accompanied by de-novo formation of blood capillaries, initiation of osteophyte formation and calcification of the articular cartilage. Blood vessel formation may be accompanied by pro-apoptotic factors that lead to chondrocytic death[8]. In the synovial membrane, new vasculature could be responsible for edema and promotion of an inflammatory process[9] which among its other effects compromise joint lubrication[10]. Vascular endothelial growth factor (VEGF) is expressed in synoviocytes[10], macrophages[11] and in chondrocytes in human osteoarthritic joints[12]. VEGF has been shown to affect chondrocytic proliferation, apoptosis, and metabolism, leading to release of metalloproteinases (MMPs), as well as other catabolic mediators that degrade the cartilage matrix[13–15]. VEGF is expressed in the superficial zone of the cartilage disc, and has been shown to be secreted from mechanically overloaded chondrocytes[13] and in OA joints *in vivo*[16]. Another study showed that synovial fluid sourced from OA patients had 20-fold higher concentrations of VEGF in comparison to its level in healthy joints, thereby suggesting that VEGF takes part in OA development[17].

The present is a study of the effect of intra-articular injection, of exogenous VEGF, on mice knee joints. The histo-morphological structure of the joints was evaluated and graded at the cellular and structural levels. OA grading of the cartilage, subchondral calcified tissues, and synovial membrane was performed.

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Materials and methods

Animals

This study was approved and conducted according to the guidelines of the Hebrew University Animal Healthcare Ethics Committee.

Male ICR (CD-1) mice 8–10-weeks-old (Harlan, Israel) were used. The mice were held in specific pathogen free (SPF) conditions at the Hebrew University Ein Kerem animal facility and were given access to conventional chow and tap water *ad libitum*.

Materials

Recombinant murine VEGF165 (PeproTec, Rocky hill, NJ, USA) was dissolved in physiological saline (Teva, Israel) to reach a concentration of 0.05 mg/μl.

Experimental design

The experimental group consisted mice that received intra-articular injections, to the synovial space (see injection procedure below), of 20 μl VEGF165 solution once a week over a period of 4 weeks. Mice were sacrificed 1,2,3,4,6 and 8 weeks post-first injection, 10 mice per time point. The effect of VEGF on OA development was examined by histological evaluation. In the control groups, 10 mice were not subjected to any kind of treatment (sham group) and 20 mice received one intra-articular injection of 20 μl of physiological saline (0.9% NaCl, Pharmaceutics Department, Hadassah Medical Center, Jerusalem, Israel) and were sacrificed 2 and 8 weeks after injection, 10 mice at each time point.

VEGF administration

Mice were anesthetized using a 0.15 μl Xylazine 2% and 0.85 μl Ketamine HCl injected intra-peritoneally (i.p.). Then the left rear knee of each mouse was shaved and the patellar ligament was exposed to allow an approach from the lateral side of the knee, using a syringe equipped with a 27 gauge needle. The needle was inserted beneath the patellar ligament, into the intra-articular space and a volume of 20 μl solution containing either saline, or VEGF was introduced.

Tissue analysis

At the above-described time points, mice were sacrificed with an overdose of the anesthesia solution injected i.p. Their rear left (injected) and right (non-injected) knees were removed, fixed in buffered formaldehyde, decalcified with a calcium-chelating agent (Calci-Clear Rapid, National Diagnostics, Atlanta, Georgia, USA), and embedded in paraffin. Sections, 6 μm thick, were cut and stained with either Hematoxylin & Eosin (H&E) or Safranin-O, according to the standard protocols (see below). The lateral and medial aspects of both the distal femoral bones and the proximal tibial bones, separated by the cruciate ligament, were examined separately. Pathological changes observed in each of the four aspects of the knee joint were graded, according to a score shown in Table I, based on the Mankin score, and modified to include bone sclerosis.

Table I
Histological scoring

	Finding	Grade
Structure	Normal	0
	Slight surface irregularity	1
	Fibrillation reaching zones 1–2	2
	Fibrillation reaching zone 3	3
	Fibrillation reaching zone 4	4
	Cracks (near the tidemark)	5
	Erosion of zones 1–3	6
	Erosion of zone 4	7
	Exposed bone	8
Cluster appearance	Normal	0
	Cluster appearance	2
Tidemark	Intact	0
	Multiple	1
	Indistinct	2
	Crossed by blood vessels	3
	None	0
Osteophyte formation	Cartilage increment	1
	Early osteophytes	2
	Developed osteophytes	4
Subchondral bone	Normal	0
	Sclerosis	1
Synovial membrane	Normal	0
	Hyperplasia – mild	1
	Hyperplasia involving half the synovial space	2
	Hyperplasia involving the entire synovial space	3
Safranin-O staining	Strong staining of the cartilage	0
	Slight reduction	1
	Moderate reduction	2
	Severe reduction	3
	No stain	4

Imunohistochemical staining of VEGF

Imunohistochemical staining of VEGF was performed on three representative knees, with scores typical for their sacrifice date, selected from each of the control and VEGF groups. Imunohistochemical staining was performed on formalin-fixed, paraffin-embedded bone sections, using the avidin–biotin–peroxidase complex method using horseradish peroxidase (HRPO)-labeled anti-mouse immuno-globulin (Histofine®Simple Stain Souse MAX-PO, Nichirei Biosciences Inc., Tokyo, Japan) and Diaminobenzidine substrate (SIGMA FAST 3,3′-Diaminobenzidine tablets, Sigma, Rehovot, Israel). Non-specific staining was avoided using incubation in 30% H_2O_2 to deactivate endogenous peroxidase and using M.O.MT.M kit (Vector Inc. Burlingame, CA, USA) according to the manufacturer instructions. VEGF staining was performed using monoclonal murine anti-human VEGF antibody (1:25 dilution, DAKO, Copenhagen, Denmark), which recognizes murine VEGF.

Antigen retrieval was performed by heat treatment of 88°C overnight before deparaffinization and incubation for 30 min in 1 μM EDTA (pH 8) after deparaffinization. Slides were counter-stained with Mayer's Hematoxylin. As a control, the same procedure was performed on a sequential section omitting the anti-VEGF antibody.

Histo-morphometric analysis

Chondrocytes number was assessed by histo-morphometry. The stained slides were analyzed using a computerized morphometric system (WinScanArray3; Galai, Migdal Haemek, Israel) connected to a light microscope (BH-2; Olympus, Tokyo, Japan). In each slide, consecutive microscope fields covering the entire articular cartilage of the examined knee were acquired (at magnification 400× and resolution of 1024 × 1024) by a color video camera (DXC-151AD; Sony, Tokyo, Japan). After acquisition, the images underwent automated light analysis and noise removal procedures to ensure color and image quality standardization in all analyses. Using PC-based Image analysis software (ImagePro+v4.5, Media Cybernetics, USA) the total area (in μm^2) of the cartilage was measured by