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QUANTITATION OF THE CHANGES IN VASCULARITY DURING ARTHRITIS IN THE KNEE JOINT WITH A DIGITAL IMAGE ANALYSIS SYSTEM.

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Introduction

Many bone and joint diseases are associated with or caused by vascular changes. Particularly osteoarthritis and rheumatoid arthritis are associated with chronic inflammatory reactions with vascular proliferation and infiltration of leucocytes. Leucocytes can release cytokines that exert a catabolic effect on articular cartilage. The effect of an inhibition of vascular proliferation by pharmaca could be that less leucocytes are able to enter the synovial fluid and that the catabolic effects on cartilage could be prevented. Quantitative methods are essential to assess the effects of such new potential inhibitors on vascularity during inflammation. The aim of this study was to analyze the number of vessels and blood perfusion simultaneously in the synovium at different time intervals after induction of arthritis with a quantitative procedure.

Materials and methods

Fourteen adult female C57Black6 mice were immunized with methylated bovine serum albumin (mBSA). Arthritis was induced by a single intra-articular injection of 60 ug mBSA. To visualize perfusion of vessels, the animals were injected intravenously via one of the tail veins with a 0.2 ml solution of phosphate-buffered saline (PBS, pH 7.4) containing 0.3 mg Hoechst 33342 (Sigma Chemical Company, St.Louis, MO), on days four and day seven after induction of arthritis. Hoechst is a fluorescent dye that is rapidly taken up by the nucleus of endothelial cells. Exactly one minute after Hoechst injection the mice were killed and both knees were quickly embedded in Tissue Tek, preventing the Hoechst dye to diffuse further into the tissues. Thereafter the knee joints were sectioned (7 um) without previous decalcification, at -22oC. Sections were stained with a monoclonal rat-anti-mouse endothelial cell marker (9F1) and with goat-anti-rat-TRITC as secondary antibody.

Immediately after the staining procedure the localization of Hoechst and the vascular marker 9F1 were analyzed with an automated digital image processing system. This system was programmed to measure synovial area (SA), vessel area (VA), area of perfused vessels (pVA), number of blood vessels (NBV), number of perfused blood vessels (NpBV), and the (perfused) vessel density $N(p)BV/SA=(p)VD$. Data were analyzed with the Wilcoxon signed rank test for the comparison between the left control knee and the right arthritic knee of each mice and for the differences between day four and seven the Mann-Whitney U test was used. All

procedures involving animals were performed with full approval of the local animal care committee.

Results

Vascular structures in the synovium were stained by the fluorescence-labeled endothelial cell marker. Nuclei of endothelial cells and cells directly adjacent to the synovial blood vessels were stained by Hoechst, thus presenting the perfused synovial vessels. Diffusion of Hoechst was restricted to a small area directly around the blood vessels decreasing the chance that adjacent non-perfused vascular structures were stained by Hoechst. The measurements showed that during arthritis the synovial area is proliferating significantly. The number of blood vessels and the number of perfused vessels in the synovium area during arthritis day four and seven had increased significantly compared to the controls, and on day seven more (perfused) vessels were present as compared to day four (Fig. 1).

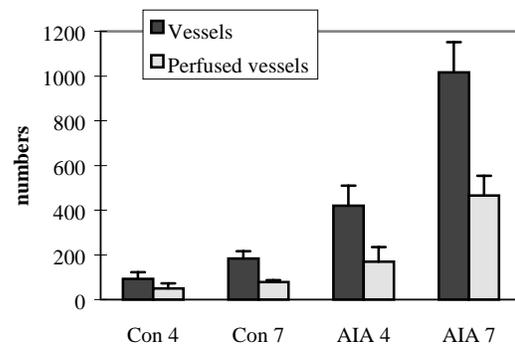


Fig. 1. Number of vessels and perfused vessels in the synovial tissue of the affected (AIA) and control (Con) knee joint on day 4 and 7 arthritis.

Discussion

The image analysis system allows analysis of whole sections of the mouse knee joint. The main advantage of this method is that both perfused and the total number of vessels can be measured in the same section quantitatively. This makes this procedure very efficient for quantitation of the effects of the different forms of drug therapy that have blood vessels as their target. **Dept Rheumatology, ***Dept Radiotherapy, University of Nijmegen.

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