MECHANICAL FACTORS AND CYTOKINES INFLUENCE THE EXPRESSION OF THE ANGIOGENESIS INHIBITOR FACTOR ENDOSTATIN IN HUMAN CARTILAGE AND FIBROCARTILAGE

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Introduction
Hyalin cartilage and fibrocartilage such as the menisci of adults are avascular with a poor repair potential.

Angiogenesis - the formation of new blood vessels from preexisting capillaries - is balanced controlled by a variety of mitogenic and chemotactic peptides that act on invading endothelial and smooth muscle cells. Endostatin is a 20 kDa antiangiogenic factor originally identified from murine hemangioendothelioma cells (1). Endostatin inhibits endothelial cell proliferation in vitro and tumor growth in vivo.

Aim of this study was to examine the spatial and temporal expression of endostatin in cartilage and fibrocartilage to gain further knowledge about the factors which might influence the regulation of angiogenesis in the musculo skeletal system.

Materials and Methods
The knee joint of human fetuses (18,23,26,32, and 34 weeks post gestation) was harvested during routine autopsy, and prepared for immunohistochemistry and the biochemical examinations. Articular cartilage and menisci were obtained from adult body donors of different age (23-76 years). Osteoarthritic cartilage (OA) was obtained during knee replacements.

Immunohistochemistry: For immunohistochemistry, the samples were immunostained with anti-endostatin (1:40 in Tris-buffered saline, 60 min; AB 1878 anti human endostatin polyclonal antibody, Chemicon, USA), or for demonstration of blood vessels by anti factor VIII (1:200; DAKO rabbit polyclonal antibody). Enzyme-linked immunosorbent assay (ELISA): Frozen tissue samples were crushed in an achate mortar under liquid nitrogen, homogenized in 150 mM NaCl 20 mM Tris/HCl-buffer, pH 7.4; a soluble fraction obtained by centrifugation (48 000 xg, 60 min), and aliquots (100 µl) were analyzed by a sandwich ELISA (Chemicon, USA Cyt274) that detects endostatin.

Stimulation of chondrocytes: Human chondrocytes were used for in vitro examinations. 10^5 cells were seeded into fresh dishes, and cultivated for 24 h in DMEM plus 10% FCS. Then, the medium was replaced by DMEM (without FCS), and the cells exposed to EGF as an example for an inflammative growth factor for 24 h. Some cells were loaded in a special cell culture chamber with intermittent hydrostatic pressure (amplitude: 0.2 MPa; frequency: 0.1 Hz; time period: 24 h / d). The cell culture chamber has been engineered in the biomechanical laboratory of the Department of Orthopedic Surgery of the Christian-Albrechts-University Kiel and has been firstly described by Hansen et al. (2). A control group was cultured without application of intermittent hydrostatic pressure. After a 24 h period of cultivation conditioned medium was withdrawn, and assayed for endostatin content by ELISA.

Statistics: Statistical significance was evaluated by the Dunnet’s multiple comparisons test or the t-test.

Results
High endostatin concentrations were found in fetal meniscus and epiphyseal cartilage. In the internal two thirds of the menisci endostatin concentrations were higher than in the periphery.

After birth endostatin concentration decreased but in articular cartilage and menisci of adults still high endostatin levels were measured. The lowest endostatin concentrations were measured in cartilage samples from patients with osteoarthritis.

Discussion
We choose endostatin as a possible inhibitor of angiogenesis in cartilage and fibrocartilage and determined its presence in the fetus, children and adults. High endostatin levels in developing cartilage and fibrocartilage reflect the angiogenic activity of fetal tissue because angiogenesis is balanced controlled by inhibiting and stimulatory peptides. This leads to the question of why angiogenesis inhibitors should be present in tissues that are angiogenic. One possibility is that the proteolytic activity that accompanies fetal growth, may also mobilize circulating angiogenesis inhibitors from precursor protein that are not antiangiogenic themselves – a mechanism that has been postulated for tumor angiogenesis (1).

In adult tissues endostatin expression is widespread downregulated but in articular cartilage and in the menisci endostatin levels were still superior. Endostatin expression in cartilage cells of suggests that the antiangiogenic potency of this molecule is critical for the avascularity of this tissue. Since formation of cartilage and fibrocartilage is a functional adaptation to compressive and shearing forces (3) it seemed likely that the avascular nature of fibrocartilage may also be influenced by mechanical stimuli. In the in vitro results of this study show that mechanical factors such as hydrostatic compressive stress are able to upregulate endostatin expression in chondrocytes. Downregulation of endostatin by inflammative cytokines (e.g. EGF) might be responsible for the low endostatin concentation found in OA cartilage. The role of endostatin in OA cartilage has to be further investigated because endostatin is a strong inhibitor of extracellular matrix degrading enzymes such as MMPs.

References
1. O’Reilly et al. (1997)
2. Hansen et al. (2001)
3. Pauwels (1960)
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Figure 1: Endostatin concentrations in cartilage

While in fetal menisci endostatin concentration were higher in the external third, during childhood and in the adult endostatin levels were superior in the avascular internal two thirds.

Figure 2: Influence of intermittent hydrostatic pressure (IHP) on endostatin secretion of chondrocytes

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