Involvement of insulin like growth factor-1 and vascular endothelial growth factor in the ossification disturbance of femoral heads in spontaneous hypertensive rats

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INTRODUCTION

It is known that the incidence of osteonecrosis (ON) and ossification disturbance were significantly higher in the femoral heads of spontaneously hypertensive rats (SHRs) than in those of Wistar Kyoto rats (WKYs). Although the pathogenesis of ossification disturbance in femoral heads in SHRs has not been elucidated, recent reports suggested that the disturbance of neovascularization and mechanical force on the weak bony structure might be possible mechanisms of deformity of the femoral head. Insulin like growth factor-1 (IGF-1) is one of the essential factors in chondrocyte function and cell differentiation in enchondral ossification. In the endothelial cells or carcinoma cells, IGF-1 is considered to have a mutual relationship with vascular endothelial growth factor (VEGF), an important growth factor that promotes the formation of blood vessels. We speculated that IGF-1 might be involved in the pathogenesis of ossification disturbance of the femoral heads of SHRs, and can regulate the expression of VEGF to inhibit vascular invasion in cartilaginous tissue. We investigated the serum and tissue levels of VEGF and IGF-1 in an SHR model to test our hypothesis. In vitro effects of IGF-1 and its inhibition by IGFBP-2 on the expression of VEGF were examined using ADTC5, which shows chondrogenic differentiation.

MATERIALS AND METHODS

In vivo study

SHRs were originally raised by selective inbreeding of WKYs. We used SHRs (n=40)(Charles River Inc., Japan) as the disease model of epiphyseal ossification disturbance of cartilage, and WKYs (n=20)(Charles River Inc., Japan) as normal control animals. All animals were used in accordance with the guidelines of Okayama University Graduate School of Medicine and Dentistry for animal experimentation. They were euthanized at 5, 10, 15 or 20 weeks (SHRs: each 10; WKYs: each 5), and the serum samples were taken and stored at –80 degrees until analysis of VEGF and IGF-1 by ELISA. Then, femoral heads of SHRs and WKYs were removed, and antero-posterior radiographs were taken by Softex (Softex co., Japan). The radiographic appearances of the femoral heads were divided into three types: type A, normal; Type B, radiolucency in the femoral head; Type C, deformity of the femoral head. After the samples were fixed and decalcified, coronal paraffin sections (3 µm) were made and stained by hematoxylin and eosin. Histological appearances of the femoral heads were scored from 0 to 2, according to the area of ossification: Stage 0, total ossification; Stage 1, partial ossification with ossification disturbance; Stage 2, no ossification. To examine the tissue levels of IGF-1 and VEGF, immunohistochemical study was performed using antibodies against VEGF (mouse polyclonal IgG;5 µg/ml), IGF-1 (rabbit polyclonal IgG;5 µg/ml).

In vitro study

A chondrogenic mouse embryonic carcinoma cell line, ATDC5 was obtained from RIKEN cell bank (Tsukuba, Japan). Cells were maintained in a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F12 supplemented with 5% fetal bovine serum and penicillin (50 IU/ml)-streptomycin (50 µg/ml). Cells were maintained at 37°C in a humidified 5% CO2/95% air atmosphere. Cells were treated by porcine insulin (10µg/ml) until they differentiated into hypertrophic chondrocytes at Day 28. After co-incubation with recombinant mouse IGF-1 (10ng/ml), recombinant mouse insulin-like growth factor binding protein 2 (IGFBP-2) (10ng/ml) for 24, 48, 72h, concentration of VEGF in the culture medium was measured by ELISA.

RESULT

Serum levels of IGF-1 and VEGF were significantly lower in SHRs than WKYs at 5, 10 weeks (p<0.002), and at 10, 15, 20 weeks (p<0.001), respectively. The incidence of histological ossification disturbance of the femoral head was markedly higher in SHRs than in WKYs at 10, 15, 20 weeks. Radiographic examination showed the number of type B and Type C femoral head was higher in SHRs than in WKYs at 15 weeks, and 20 weeks, respectively. The serum levels of VEGF and IGF-1 had statistically significant relationships with histological ossification disturbance and radiographic changes. Immunohistochemistry showed that high populations of VEGF and IGF-1 positive chondrocytes were detected mainly in WKY at 5, 10 weeks. In contrast, the positive reaction was significantly lower in chondrocytes in the femoral heads of SHR at 5, 10 weeks.

In vitro study using ADTC5 cells demonstrated that treatment by IGF-1 increased the expression of VEGF by ATDC5 cells in a time-dependent manner. Blocking of IGF-1 by IGFBP-2 decreased the VEGF expression by ADTC5 cells.

DISCUSSION

SHRs are a known model of ossification disturbance of Perthes’ disease. Several studies have reported delayed expression of serum levels of IGF-1 during the development of SHR. Lower levels of IGF-1 in the serum are also reported in patients with Perthes’ disease. In the endothelial cells or carcinoma cells, IGF-1 promotes VEGF, which is an essential growth factor involved in neovascularization, vascular invasion, and activation of osteoprogenitor cells. In the current study, we first demonstrated the serum levels of IGF-1 as well as VEGF had close relationships with the radiographic and histological stages of ossification, expression and localization of VEGF in the femoral head during the development of SHRs. The in vitro study revealed that the expression of VEGF was regulated by IGF-1 in the hypertrophic ATDC5 cells. These results suggested that lower levels of IGF-1 in the serum can regulate the local expression of VEGF by chondrocytes, and possibly osteoprogenitor cells or endothelial cells to suppress the angiogenesis and subsequent ossification in femoral heads of SHRs.