Expression of Vascular Endothelial Growth Factor During Development of Steroid-induced Osteonecrosis: An Experimental Study in Rabbits

*Sinichi Yagishita; *Katsuro Tomita; *Takeshi Horii; + **Tadamichi Matsumoto; **Mitsuru Tsuchiya; + **Takayuki Etoh; ***Osamu Amano; + ***Shoichi Ikeda; Kanazawa University, Department of Orthopaedic Surgery, Kanazawa, Japan; + **Kanazawa Medical University, Department of Orthopaedic Surgery, Ishikawa, Japan

Abstract Introduction:
Vascular endothelial growth factor or VEGF is an angiogenic promoter that is rapidly induced as a response to local hypoxia. The up-regulation of VEGF has been validated in several animal models, such as cerebral, myocardial or limb ischemia. Recently, the interpretation that the steroid-induced osteonecrosis or SION of the femoral head may be caused by an acute ischemic event is becoming increasingly acceptable. Therefore, it appears important to clarify the association between SION and ischemia to investigate the expression of VEGF in SION of the femoral head. In this study, we investigated VEGF expression in a rabbit experimental model in order to determine the onset of the ischemic events in SION. Enter the body of your paper here in two-column format.

Methods:
Mature Japanese White rabbits were divided into 6 treatment groups and an untreated control group. Rabbits in treatment groups received a single intramuscular injection of 4 mg/kg of methylprednisolone acetate. Rabbits that belonged to one day, three day, five day, one week, two week, and four week groups were euthanized at one day, three days, five days, one week, two weeks, and four weeks after initial steroid treatment, respectively. After steroid treatment, tissue samples were obtained by dissection and removal of the femurs from both sides of the animals. The development of osteonecrosis was examined histopathologically after HE staining. The criteria defined by Yamamoto et al. were used to determine the presence of osteonecrosis as shown on this slide. The expression of VEGF was examined by immunohistochemistry. If ten or more of the cells expressing VEGF had aggregated, we defined this state as “VEGF positive”. After extracting bone marrow samples from the proximal femurs, the expression of VEGF-mRNA was examined by Northern blot analysis and the production of VEGF protein by Western blot analysis. Using these methods, we examined the development and prevalence of osteonecrosis after steroid treatment, the histopathological features and the location of VEGF positive cells, and the time-course of changes of VEGF-mRNA and VEGF protein expression.

Results:
On macroscopic histopathological findings, osteonecrosis was observed in the bone marrow between intertrochanteric and subtrochanteric areas. On microscopic examination, the osteonecrotic area had increased eosinophilic changes, when compared with the surrounding bone marrow tissue, and became clearly distinguishable from the surrounding normal bone marrow. Osteonecrosis was not observed in the untreated controls, one-day or three-day treatment groups. The earliest indication of osteonecrosis was five days after the steroid treatment, and the prevalence of osteonecrosis was almost equal seven days after the steroid treatment. VEGF positive cells tended to be located in the medial proximal part of the femur between metaphysis and diaphysis, where osteonecrosis was frequently observed in the five day, and one-week groups. So VEGF expression was accompanied by development of osteonecrosis. Northern blot analysis demonstrated the single dominant band, about 3.9 kilobases in length, in all the groups. This corresponded to the size of VEGF-mRNA. The peak of VEGF-mRNA expression occurred 3 days after the steroid treatment. The amount of VEGF-mRNA expression was 2.1- and 4-fold greater than that of the untreated control. This increase was statistically significant. VEGF-mRNA expression decreased gradually after its peak and became almost equal to that of the untreated controls one week after the steroid treatments. The peak of VEGF protein production, as determined by using Western blot analysis, occurred 3 days after the steroid treatment.

Discussion:
Immunohistochemical features included that the VEGF positive cells tended to be located in the medial proximal part of the femur in the three day, five day, and one week groups before osteonecrosis was observed, however, these positive cells were not located in the area of osteonecrosis after osteonecrosis was observed. On this basis, it is suggested that VEGF production was switched on as a result of the ischemic event that causes osteonecrosis. The peaks of VEGF-mRNA expression and VEGF protein production occurred 3 days after the steroid treatment. Considering that the earliest indication of osteonecrosis was five days after the steroid treatment, and that the prevalence of osteonecrosis was almost equal after seven days, our results suggest that the onset of the ischemic events that caused osteonecrosis occurred approximately 2 days after the initial steroid treatment. That we found a single peak of VEGF-mRNA expression occurring 3 days after the steroid treatment supports the hypothesis that osteonecrosis in steroid-treated rabbits was caused by a single acute ischemic event.

Affiliations of Institutions for Co-authors:
***Kanazawa University, Department of Anatomy (I), Kanazawa, Japan

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