ABSTRACT INTRODUCTION:

The association between steroid administration and the development of osteonecrosis has been well established since 1957. However, the precise pathogenesis of osteonecrosis remains unknown. Investigations on the mechanism underlying the pathogenesis of osteonecrosis have so far focused on either diminished blood supply to bone or marrow cell differentiation.

Peroxisome proliferative activated receptor, gamma; PPARγ is a member of the nuclear hormone receptor subfamily of transcription factors, and this forms in PPARγ-1 and PPARγ-2. PPARγ-2 protein stimulates the differentiation of the adipocyte, and is relevant to the bone’s metabolism. Previous studies proved that the increase amount of the activation in the PPARγ-2 gene stimulates the differentiation of adipocytes, and adversely restrains the differentiation in the osteoblasts. Bone morphogenetic proteins (BMPs) belong to the family of the transforming growth factor-beta (TGF-β). The major function of the BMP is to promote the bone’s development, formation and recovery. The BMP-2 plays a significant functional role in this process. Via the bone morphogenetic proteins receptor, type II, BMP-2 transfers the messages by the smad signaling pathway to stimulate the cell differentiation of the mesenchymal stem cells to form the osteoblasts.

In recent years, a number of researchers have proposed some important roles played by PPARγ-2, BMP2 and BMPR2 in steroid-induced osteonecrosis. However, the underlying mechanism has yet to be elucidated. The primary objective of this study was to explore the correlation between PPARγ-2, BMP2 and BMPR2 expression and osteonecrosis (ON) in systemic lupus erythematosus (SLE) patients.

METHODS:

Samples of whole blood were obtained from the SLE patients at the Division of Rheumatology, Immunology and Allergology of our Hospital. This research includes 220 SLE patients, 55 out of 220 had the ON, but 165 out of 220 had no ON. All of these patients have taken the corticosteroid medication for more than two years without taking the antithrombosis medicine, and having no alcoholism behaviour. Peripheral blood was collected from the subjects for analysis of PPARγ-2, BMP2 and BMPR2 mRNA expression.

Total RNA was isolated from tissue peripheral blood specimens with QIAGEN® RNA Blood Mini Kit (QIAGEN, Valencia, CA) according to manufacturer's instructions. Poly(A)+-enriched RNA was purified from total RNA using Dynabeads® mRNA DIRECTTM kit (Dynal AS, Oslo, Norway). First-strand cDNA was synthesized from total RNA by using the Advantage RT-for-PCR kit (Clontech, Palo Alto, CA) by following procedures described in the manufacturer's technical manual.

Real-time PCR was performed in a Rotor-Gene 2000 thermocycler (Corbett Research, Inc.). The reaction mixture contained 2 μl of 20 mM dNTP, 2 μl of 30 mM MgCl2, 2 μl of 20X SYBR green, 2 μl of 1 μM forward primer, 2 μl of 1 μM reverse primer, 4 μl of nuclease free water, 2 μl of 80–100 ng/ml cDNA, and 2 μl of 1 U/μl polymerase. PCR conditions were as follows: 35 cycles of denaturation at 95°C for 20s, annealing at 60°C for 20s, and extension at 74°C for 40s. PCR products (i.e. synthesized dsDNA) were quantified by measuring the fluorescent intensity at the end of each amplification cycle. For each sample, real-time PCR analysis was repeated in three independent experiments to ensure the reproducibility of results.

All data was analyzed using the Statistical Package for the Social Sciences Ver. 10.0 software (SPSS Inc., Chicago, IL, USA). Results were expressed as a mean ± standard error. The chi-square and the student’s t tests were used to compare the clinic-pathologic parameters between the genes up and down expression. A probability of less than 0.05 was considered to be statistically significant.

RESULTS SECTION:

In the peripheral blood of these 220 SLE patients, 55 patients had the osteonecrosis resulting from taking the steroid medicine for 4 to 5 years. The remaining 165 patients did not develop osteonecrosis despite being treated with steroid medicine for over 9 years. In all subjects, more than 90% of the patients are female, and less than 10% of the patients are smokers.

Having collected the patients’ peripheral blood for the total RNA extraction and reversed transcription of the cDNA. The mean fold changes of PPARγ-2, BMP2 and BMPR2 mRNA in SLE with ON are 0.83±0.38, 1.15±0.31 and 1.28±0.12; compare with 0.31±0.06, 1.29±0.12 and 2.23±0.22 in non-ON patients. PPARγ-2 mRNA in SLE with ON was significantly higher than that in non-ON (P = 0.044). Furthermore, BMPR2 mRNA was significantly lower than that in ON (P=0.036), but there was no differences in BMP2 mRNA (P=0.659).

DISCUSSION:

In this study, we compare the results between the steroid treated SLE patients who experiencing ON and non-ON symptom, and comparing the amount of the activation of PPARγ-2, BMP2 and BMPR2 mRNA in the peripheral blood, the results showed that the PPARγ-2 mRNA expression level of the SLE patients with ON increases 37%, but the BMPR2 mRNA’s activation decreases 57%. Moreover, there were no significant differences of the BMP2 mRNA’s activation in both groups.

BMP2 can induce MSCs to differentiate and form the osteoblasts, an important factor to the bone’s growth and recovery. BMPR2 encodes a member of the bone morphogenetic protein (BMP) receptor family of transmembrane serine/threonine kinases and it also accepts signals from BMP2 through the Smad signaling pathway and transmits to RUNX2. It has been shown in previous experiment that after treating Multipotential mesenchymal cells (D1) with Dexamethasone, the RUNX2/cbfα1 genes expression would decrease.1

PPARγ-2 has been recognized as an adipocyte-specific transcription factor which is important for the mesenchymal stem cells to differentiate and form adipocytes. In the previous studies, it has been proved that the Dexamethasone can stimulate the PPARγ-2 gene’s fold change to increase, and the same result was also shown in this experiment.2

In the prior studies,2 we demonstrated that in vivo and in vitro animal studies, dexamethasone stimulates bone marrow mesenchymal cell to differentiate into adipocytes as well as the accumulation of fat in the marrow, while suppressing cells from differentiation into osteoblasts. Therefore, the steroids would stimulate the increase of the fold changes of the PPARγ-2 gene in the MSCs causing the increase of adipogenesis. Moreover, this process might also lower the activation of BMPR2 causing the signal transmission of BMP2 to decline, and also influence the activation of RUNX2 to cause the decline of osteogenesis. Since adipocytes and osteoblasts share a common pool of progenitor cells. Therefore, when the MSCs preferentially increase the adipogenesis, and this process adversely restrains the differentiation of osteogenesis. Hence, upset the microenvironment of the bone marrow and impaired the property of the osteoblasts for bone remodeling, or repair of the necrotic bone. Ultimately, the osteonecrosis developed. We also suspect these who developed ON after steroid treatment might preferentially have higher susceptibility and this process could be detected using peripheral blood before or after the drug was instigated. Despite that the detailed molecular mechanism of steroid-induced osteonecrosis has not yet been identified.

REFERENCES: