Effects of Erythropoietin and GCSF on Traumatic Osteonecrosis of the Femoral Head in Rabbits

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Introduction: Osteonecrosis, also called avascular necrosis (AVN), affects predominately young males in their 20s-50s. This disease can be idiopathic or secondary to trauma, steroids, excessive alcohol use, systemic lupus erythematous, and hemoglobinopathies. Osteonecrosis of the femoral head (ONFH) is initially asymptomatic, and may lead to collapse of the femoral head with subsequent hip joint destruction ultimately requiring total hip arthroplasty (THA). Due to the young age of these patients, patients with AVN may need multiple surgeries in their lifetime. Thus, methods to prevent femoral collapse from AVN are desirable to prolong the need for THA.

Several cellular modulators have been studied for their healing properties. Erythropoietin (EPO) has been suggested to be beneficial in repairing ischemic damages due to its angiogenic properties. Furthermore, some studies have suggested that Granulocyte colony-stimulating factors (G-CSF) may also have some benefit through inducing bone marrow stem cell mobilization. Considering the reperfusion effects of EPO in ischemia and preventive properties of GCSF, this study aims to investigate the potential effects of EPO, and EPO with G-CSF on traumatic avascular necrosis of the femoral head in rabbits.

Methods: A randomized double-blinded study was performed in 18 rabbits. All 18 rabbits were female and weighed between 3.0-3.5kg. After receiving anesthesia, all rabbits underwent traumatic osteonecrosis of the femoral head through periosteal stripping of the neck, transection of the ligamentum teres, and ligation using nylon through a lateral incision.
procedure, each rabbit received intravenous cefazolin 25mg/kg to prevent complications from infections. Confirmation of successful induction of ONFH on each of the rabbits was accomplished by 3D computed tomography angiography (CTA) of the hip following the procedure.

The 18 rabbits were subsequently divided into three groups of 6 each; EPO only, both EPO and G-CSF, and a control group. In the EPO only group, the rabbits received a single administration of intra-peritoneal EPO (5000 IU/kg) immediately following the procedure. The G-CSF and EPO only group of 6 rabbits received daily subcutaneous (SQ) injections of 300 μgr/kg of GCSF treatment for 5 days following the single administration of intra-peritoneal EPO (5000 IU/kg).

At the second and fourth weeks after surgical induction of ONFH, femoral heads (three rabbits from each group at week 2 and the remaining three rabbits from each group at week 4) were put under anesthesia and sacrificed for histologic examination. Three sections were taken from each femoral head including a subchondral, central (widest section of the head), and between the aforementioned zones. The samples were immediately fixed in 10% formalin, decalcified with acetic acid, and then paraffin embedded. The prepared samples were then cut into 5 µm sections, mounted on slides, and stained with haematoxylin & eosin. A standard light microscope was used to evaluate 10 high power fields of each section. Bone necrosis was measured as the percentage of empty lacunae. Empty lacunas surrounded by full ones were not considered empty, because they were considered to be an artifact of
sectioning. Overall necrosis was the sum of bone necrosis with surrounding bone marrow cell necrosis. Statistical analysis was performed using one way ANOVA, post hoc test, and independent samples t-test. An alpha level of 0.05 was used to determine significance.

**Results:** The initial two-week period comparison of necrosis between each groups revealed 50% necrosis in the control group, 40% necrosis in the EPO group, and 60% necrosis in the EPO with G-CSF group (p=0.125). For bone necrosis, the two-week analysis revealed 40% of empty lacunae in the control group, 27% in the EPO alone group, and 33% in the EPO with G-CSF group (p=0.171). Although there was a trend toward more necrosis and empty lacunae in the control group compared to either EPO alone or EPO with G-CSF, no difference could be demonstrated.

At the 4-week checkpoint, the comparison of necrosis in each group showed 27% necrosis in the control group, 10% of necrosis in the EPO group and 25% necrosis in the EPO with G-CSF group (p=0.411). Additionally, bone necrosis was observed in 27% of empty lacunae in the control group, 10% in the EPO group, and 25% in the EPO+G-CSF group (p=0.365).

EPO also showed to improve the regeneration through time. The comparison of the evaluated samples at the second and fourth week within the EPO groups indicates that there was both less necrosis (40% vs. 10%; p=0.017) and empty lacunae (27% vs. 10%; p=0.05). Also comparing the percent necrosis and empty lacunae after 4-weeks in the EPO group with the 2 week control group showed statistically significant reduction in both necrosis and empty lacunae(p=0.048). However, the EPO with G-CSF group did not show statistical reduction in comparison with the control group at 2nd and 4th week. (p=0.07 & p=0.58, respectively)

**Discussion:** The results of our study demonstrate that erythropoietin may reduce the necrosis from post-traumatic ONFH in rabbits. This study supports the findings of Chen et al, which almost demonstrated that EPO decreased the incidence of osteonecrosis, assessed histologically, in steroid-induced ONFH in rat models. Unlike some studies that have reported improved avascular necrosis outcomes with G-CSF, our results found no improvement in outcomes, which may have occurred because G-CSF was administered with EPO. G-CSF may have further increased the hematocrit values leading to hyperviscosity syndrome and reduced bone perfusion. Limitations of this study includes not administering G-CSF alone and the small sample size, which may limit the power of this study. In light of these issues, EPO administration following traumatic ONFH demonstrated promising results that warrants further investigation with increased sample sizes. It remains unknown whether the EPO effects on ONFH can be applied to humans. Future studies should elucidate the role of EPO administration and other drugs for AVN prevention.

**Significance:** In rabbits with traumatic AVN of the femoral heads, EPO was demonstrated to show reduced bone necrosis compared to the control groups but not when co-administered with G-CSF. Thus EPO may have important clinical applications since it potentially may delay femoral head collapse and, ultimately, total hip arthroplasty in young AVN patients. Given these findings, future studies should investigate the ideal dosage and applications of EPO on other sources of AVN, including the most common cause of AVN, idiopathic.

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