Research Article

JBS Journal

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

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**Abstract**

**Introduction**: To assess the effects of Erythropoietin and Granulocyte colony-stimulating factor on traumatic osteonecrosis of the femoral head in rabbits.

**Materials and Methods**: 18 female rabbits were induced with traumatic osteonecrosis of the right femoral head by standardized procedure then were divided into three groups: (1) administered post-op 5000 IU/kg intraperitoneal erythropoietin, (2) administered post-op 5000 IU/kg intraperiotneal erythropoietin and daily SC injections of 300 µgr/kg of GCSF for 5 days, and (3) control group. The histologic comparisons of necrosis and empty lacunae of the three groups were compared at weeks 2 and 4.

**Results**: After 2 weeks in the EPO group showed 40% necrosis and 27% empty lacunae, whereas after 4 weeks, showed 10% necrosis and 10% empty lacunae($p<0.05$ and $p=0.05$, respectively). 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(10% vs. 50%) and empty lacunae(10% vs. 40%)($p<0.05$).

**Conclusion**: In conclusion, the intraperitoneal administration of single dose EPO can effectively prevent the amount of necrosis in rabbit model of ONFH after 4 weeks.

**Keywords:** Erythropoietin, Granulocyte colony-stimulating factor, AVN, femoral head, Orthopaedics, A.6, A.9, A.10

## Introduction

Osteonecrosis, also known as avascular necrosis (AVN), is a disease that may affect different bones as a result of temporary or permanent loss of the blood supply. The femoral head is most commonly affected by this disease and affects predominately men in their 20s-50s.[1,2] The disease can be idiopathic or secondary to an underlying systemic disease. Some well known secondary etiologic factors are trauma, steroid intake, excessive alcohol use, systemic lupus erythematosus, and hemoglobinopathies.[3]

Osteonecrosis of the femoral head (ONFH) is initially asymptomatic, and if left untreated, more than 70% lead to collapse of the femoral head with subsequent hip joint destruction.[4] In this stage, the only definite treatment is total hip arthroplasty.[5] Unfortunately, the results of arthroplasty in these patients have been less than satisfactory, with failure rates ranging from 10% to 50% at the 5-year follow-up.[6]

With the average age of patients with ONFH at 35, which is significantly younger than the average age of patients with osteoarthritis, these patients tend to be more

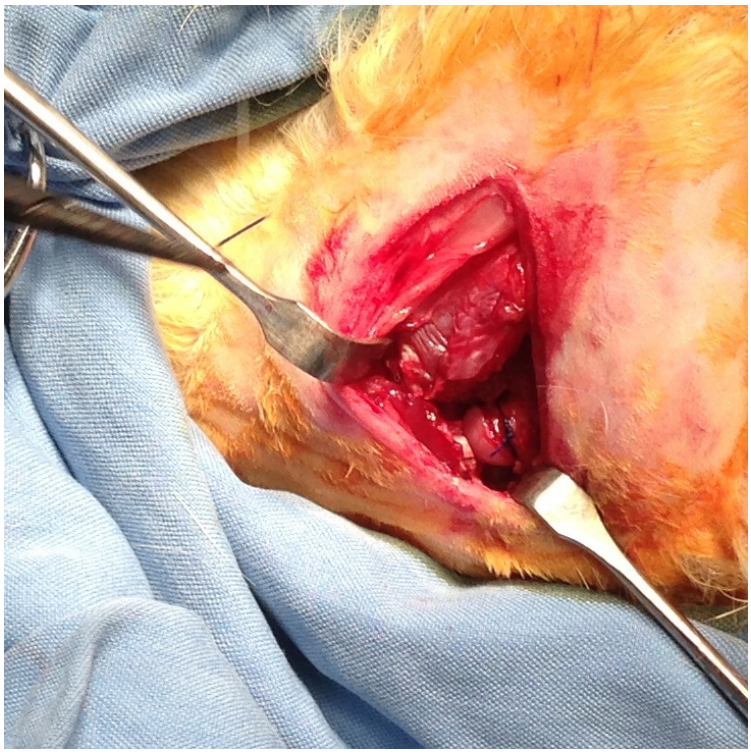

**Figure 1.** The technique of inducing traumatic AVNFH in rabbit. Following dislocating the femoral head, the periosteum of femoral neck is detached and a nylon suture is tied around the neck.

active and resulted in higher rates of revision.[7,8,23,25] Chandler *et al.* presented that in patients less than 30 years old undergoing total hip arthroplasty resulted in significant increase in prosthesis loosening due to their active lifestyles. And despite improvements in the design and technique of hip arthroplasty, it is unlikely that prosthetic hips will last more than the 40 years life expectancy for these patients.[9,10]

Therefore, the total hip arthroplasty should be delayed as long as possible. However, hip salvaging procedures, both surgical and non-surgical, such as core decompression, bone grafting, osteotomies, electrical stimulation, and pharmacologic agents have shown to be relatively ineffective in preventing the progression of the disease.[11,12]

Several cellular modulators have been studied for their healing properties.[5] Erythropoietin (EPO) has been suggested to be beneficial in repairing ischemic damages due to its angiogenic properties.[13] EPO is a hormone that is produced by the kidneys to stimulate red blood cell production, and has been used to treat anemia, especially in the setting of end-stage renal disease. Recent studies have shown alternative uses for EPO.

EPO has been shown to promote wound healing by inducing angiogenesis either directly or via vascular endothelia growth factor(VEGF).[14] EPO has also been shown to have bone regeneration/ formation properties.[15,16] Additionally, Granulocyte colony-stimulating factors (G-CSF) has shown to have benefit in preventing further progression of ostenoecrosis by inducing bone marrow stem cell mobilization.[17,18]

Considering the reperfusion effects of EPO in ischemia and preventive properties of GCSF, this study aims to investigate the potential effects of induction of angiogenesis in patients with ONFH by EPO alone and in

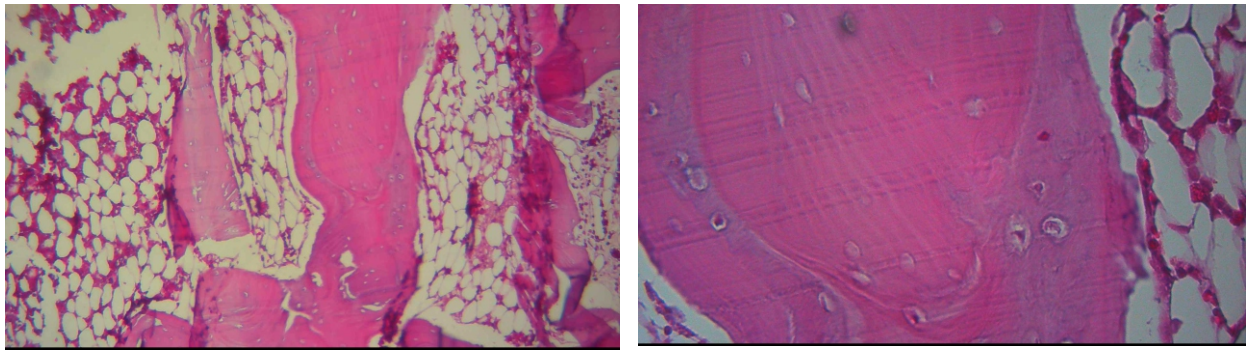

**Figure 2.** Determination of the empty lacuna percent: During microscopic examination of trabeculae, 10 field are selected randomly and in all fields, 50 lacunae, whether occupied or empty, are counted. Then the percent of empty lacunae is calculated.

combination with GCF using rabbit models.

## Materials and Methods

### *Animal Model Study*

This study is a double-blinded study using 18 female rabbits. All 18 rabbits underwent induced traumatic ONFH on the right hip. The induced traumatic ONFH was achieved by periosteal stripping of the neck, transecting ligamentum teres, followed by ligation using nylon.(Figure 1) After the procedure, each rabbit received intravenous cefazolin 25mg/kg to prevent complications from infections.

Rabbits are placed in three groups of 6. In the first group, rabbits received a single administration of intra-peritoneal EPO (PDpoetin, Pooyesh Darou, Iran) (5000 IU/kg) following the procedure. In the second group, rabbits received daily subcutaneous(SC) injections of 300 µgr/kg of GCSF (PDgrastim, Pooyesh Darou, Iran) treatment for 5 days after the single administration of intra-peritoneal EPO (5000 IU/kg). The third group served as a placebo control group, the injected doses were replaced by placebo. At the second and fourth weeks, femoral heads (three rabbits from each group at week 2 and the remaining three rabbits from each group at week 4) were removed and sent for histologic examination.

Samples for histologic examination were fixed in 10% formalin and acetic acid and then were placed in paraffin. The prepared samples were then cut in 5-micron sections and stained with H & E. They were evaluated by a standard light microscope. Yammamoto *et al.* presented histological diagnostic criteria for ONFH in a rabbit model by the presence of hematopoietic cell necrosis (cytolysis, karyorrhexis, or karyolysis), fat cell necrosis (the loss of either nuclei or distinct cell borders), and osteonecrosis. Osteonecrosis was assessed based on histopathologic alterations of bone necrosis (the presence of empty lacunae or pycnotic nuclei of osteocytes and bone marrow cell necrosis.

The statistical analyses were obtained by one way ANOVA, post hoc test, and independent samples t-test. $P<0.05$ was considered significant.

## Results

The rabbits used in this study were 18 female rabbits weighing 3.0-3.5 kg. The initial comparison of necrosis between each group at the two-week period showed 50% of necrosis in the control group, 40% necrosis in the EPO group, and 60% necrosis in the EPO+G-CSF group ($p<0.05$).(Table 1)

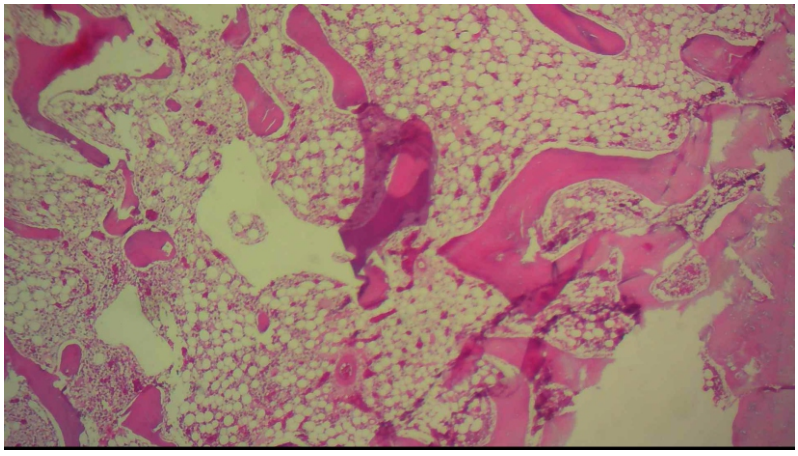

**Figure 3.** Determination of the femoral head necrosis in H&E staining: Presence of hematopoietic or fat cells necrosis or empty lacunae or condensed nucleolus in osteocytes shows the femoral head necrosis.

The comparison of empty lacunae at this point stated 40% of empty lacunae in the control group, 26.6% in the EPO alone group, and 33.3% in the EPO+G-CSF group ($p>0.05$). Although underpowered, there was a trend of more necrosis and empty lacunae in the control group compared to either EPO alone or EPO+G-CSF. (Table 1)

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+G-CSF group ($p>0.05$).(Table 2) Additionally there was 26.6% of empty lacunae in the control group, 10% in the EPO group, and 24.6% in the EPO+G-CSF group ($p>0.05$). These results indicated that the EPO group had statistically less necrosis and empty lacunae compared to both the controlled group and EPO+G-CSF.(Table 2)

EPO also showed to improve the regeneration through the time. The comparison of the evaluated samples at the second and fourth week within the EPO groups indicates that there was less necrosis (40 vs. 10; $p<0.05$) 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($p<0.05$). However, the EPO+G-CSF group did not show statistical reduction in comparison with the control group at 2$^{nd}$ and 4$^{th}$ week. ($p>0.05$ & $p>0.05$, respectively)

**Table 1.** Pathologic findings after 2 weeks.

| Case | | Control | EPO Group | EPO/GCSF Group |
|---|---|---|---|---|
| 1 | % of Necrosis | 50% | 50% | 60% |
| | % of Empty Lacuna | 48% | 30% | 28% |
| 2 | % of Necrosis | 60% | 30% | 70% |
| | % of Empty Lacuna | 40% | 28% | 44% |
| 3 | % of Necrosis | 40% | 40% | 50% |
| | % of Empty Lacuna | 32% | 22% | 28% |

## Discussion

Comparing the results at 4-week period in the EPO group with the control group, a favorable outcome in preventing progression to necrosis in post-traumatic ONFH in rabbits was found. Furthermore, within the EPO group samples had significantly less necrosis at the 4th week in comparison with the 2nd week.

Wu *et al*. presented significant decrease in incidences of ONFH in rabbit models using G-CSF with stem cell factor(SCF). Our results may differ because our study used a different adjunctive therapy using EPO with G-CSF rather than (SCF) with G-CSF. In our study, the EPO+G-CSF group had worse outcomes than the EPO group, which may be due to the higher blood cell count in this group. High hematocrit values are known to adversely affect red blood cells properties19. Elevated hematocrit levels are associated with down-regulation of nitric oxide synthesis, activation of tissue endothelin system and increased in situ thrombosis, which can lead to reduced tissue perfusion.[20]

Chen *et al.* found that using EPO on steroid-induced ONFH in rat models had a positive effect in reducing the incidence of osteonecrosis. They have shown that daily EPO administrations of 500U/kg for 1 week following steroid-induced ONFH showed marked decrease in the incidence of ONFH. The analysis of TUNEL assay showed inhibition of apoptosis of osteoblasts and osteocytes, while increasing the expression of VEGF. This study supports the findings of our study and shows beneficial effects of EPO in non-trauma induced ONFH.

Some limitations of this study were noted. Firstly, the small sample size may limit the power of this study. Secondly, in addition to checking necrosis and empty lacunae, checking the amount of apoptosis with TUNEL staining would have allowed for a more accurate representation of necrosis and its pathophysiology. Lastly, measuring the blood cell count in each group would have allowed us to determine the possible adverse effects of hematocrit in EPO+G-CSF.

As this is the first study on EPO administration in ONFH of rabbits, we recommend studies with different doses of EPO, especially low doses given in a longer period. In isolated newborn rabbit hearts, EPO concentrations of 0.5 to 5.0 U/ml improved recovery from cardiac ischemia, whereas higher concentrations of 10.0 U/ml had no effect.[21] Also, repeated high doses of EPO has shown to aggravate left ventricular dysfunction in rat models of myocardial infarction.[22] In addition, as Bakhshi *et al.* has hypothesized, local delivery of EPO during core decompression may enhance bone regeneration in ONFH. A further study of administering EPO in conjunction to a surgical method of preventing progression of ONFH may show benefit.

**Table 2.** Pathologic findings after 4 weeks.

| Case | | Control | EPO Group | EPO/GCSF Group |
|---|---|---|---|---|
| 1 | % of Necrosis | 40% | 5% | 5% |
| | % of Empty Lacuna | 32% | 4% | 6% |
| 2 | % of Necrosis | 20% | 20% | 50% |
| | % of Empty Lacuna | 20% | 20% | 48% |
| 3 | % of Necrosis | 30% | 5% | 20% |
| | % of Empty Lacuna | 28% | 6% | 20% |

In conclusion, the intraperitoneal administration of single dose EPO can effectively reduce/prevent the amount of necrosis in rabbit models of ONFH after 4 weeks. Further studies with larger sample size, varying EPO dosage, and adjunctive therapy are indicated at this time. ■

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**Acknowledgments**

The Authors express their appreciation to Hadi Ghoraishian, Afshin Bahrami, Poopak Farnia for their great support and cooperation.

**Financial Disclosure**

None declared.

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