Title:

Effect of Zoledronic Acid and Vitamin E on Surgical–Induced Femoral Head Osteonecrosis in Rabbit

Abstract:

Objectives: Femoral head osteonecrosis is a progressive disease with disabling outcomes in hip joint if not treated. This study was designed to compare the effects of zoledronic acid plus vitamin E versus zoledronic acid alone in femoral head osteonecrosis induced by surgical method in rabbits.

Methods: 26 Japanese white adult male normal rabbits at 28-32 weeks old were underwent surgical femoral dislocation to devastate the femoral neck vessels; the femoral neck vessels were ligated. Next, the first 10 rabbits received Zoledronic acid alone that was injected at the 1st and the 4th week; the second group (10 rabbits) received the zoledronic acid that was injected 1st and the 4th week plus oral vitamin E that was added to the food(daily) for 12 weeks and the third group was considered as control group. The treatment process was persisted for 12 weeks and the radiographic and postmortem pathological assessments were performed. The Ficat classification, Epiphyseal Quotient, new bone formation, and residual necrotic bone were assessed. The results were analyzed by statistical chi-square, paired t-tests, independent t-test, Fisher's test and p-value < 0.05 was considered significant.

Results: There were significant statistical difference between the combination therapy group with control group for Ficat at 12^{th} weeks (p-value=0.048) but there was no significant statistical difference between monotherapy and combination therapy groups for Ficat at 12^{th} weeks. Also, both treated groups with control group had significant statistical difference for RNB (p-value=0.015). There were no statistically significant differences between three groups

for Ficat at 6th week, EQ at 6th and 12th week and NBF (p-value ≥ 0.05) in comparing the three groups.

Conclusion: Although zoledronic acid therapy along with vitamin E could improve some radiologic and pathological indices related to femoral head osteonecrosis, vitamin E showed relative impact.

Running title:

Combination therapy of Zoledronic acid and Vitamin E on Femoral Head Osteonecrosis

Keywords: Zoledronic acid, Vitamin E, Femoral head osteonecrosis

Introduction:

Femoral head Osteonecrosis is known as a progressive disease (1). Lack of on time treatment leads to the collapse of femoral head (2). It is estimated that 20,000 to 30,000 new cases are diagnosed with osteonecrosis annually in the United States (3). Trauma or slipped capital femoral epiphysis (SCFE) at adolescence both can be associated with an increased risk of Femoral head Osteonecrosis (4, 5). Besides trauma, there is a long list of conditions that may reason or are related to Femoral head Osteonecrosis including steroids utilization, alcohol abuse and etc.; however, some of the cases are considered idiopathic (1, 5-7). Femoral head Osteonecrosis may be painless at early stage; then, with progression of the disease, it may be followed by an unexpected beginning of severe pain at groin, and the painful limitation of hip motion is the eventual presentation (1,2). Eventually, collapse and fracture of the femoral head, leading to end-stage degenerative changes may be expected (1, 8). Accordingly one of the goals in treatment of femoral head osteonecrosis is prevent the progression specially prevent the collapse.

Complete evaluation of the patient's history and physical exam is required to diagnose the Femoral head Osteonecrosis Plain radiographs have been for years the basic imaging

assessment for recognition and staging of Femoral head Osteonecrosis though bone scan demonstrates high sensitivity for early detection (5). It is considered that MRI is the method of choice for detecting and staging of the Femoral head Osteonecrosis (5).

There is a lot of knowledge about the factors that trigger avascular necrosis of bone but little agreement; the intraluminal obliteration and the extraluminal obliteration are the possible involved mechanisms; also, cytotoxicity and genetic factors may be implicated (7). In detail, intraluminal obliteration of blood vessels can occur by microscopic fat emboli, sickle cells, nitrogen bubbles (caisson disease), or focal clotting due to procoagulant abnormalities and the extraluminal obliteration may result from rise of marrow pressure or increased marrow fat (7). It seems that the process of avascular necrosis of bone is multifactorial even though the final mechanism is always critical ischemia (7).

Perhaps due to incomplete knowledge about the events of avascular necrosis of bone, there is not a gold standard treatment for femoral head osteonecrosis and a multidisciplinary approach is usually necessary (3). The therapeutic approaches consist of pharmacologic agents, biophysical treatments, and surgical treatments (3).Despite various non operative treatment options the most favorable results were seen by surgical intervention.Trend of orthopedic surgeons and their most common decision in current practice is surgery; majority of them consisting a core decompression part.

In this regard, many investigations on animal models are going on to evaluate the various pharmaceutical or surgical therapeutic methods. Several studies have been designed to evaluate the usage of various medications as enoxaparin and lovastatin, nitrate patch, rifampicin, vitamin E, bisphosphonates, sodium ferulate and ACTH (9-17). Among these therapeutic agents, bisphosphonates including alendronate and zoledronic acid seem effective in treatment of femoral head osteonecrosis and Legg-Calve´-Perthes disease-like in rat models, respectively

(13-15, 18). Furthermore, the animal model investigations revealed that bisphosphonate therapy could improve bone volume and mineral density and preserve the femoral head shape better (13). In addition, there is evidence that oral administration of vitamin E (α-tocopherol) at a safe dose to rabbits (in their food) can suppress corticosteroid-induced osteonecrosis (12).

Therefore, we intended to evaluate the effect of combination therapy of zoledronic acid and vitamin E and solitary zoledronic acid therapy on surgical–induced femoral head osteonecrosis in rabbit.

Materials and Methods:

Experimental animals. 26 healthy Japanese white adult male rabbits at 28-32 weeks old (weight, 2-4.250 kg) were purchased from Pasteur Institute of Iran. The animals were and raised in separate cages on a 12/ 12-hour light/dark cycle and 23±2^{o}C temperature, fed with a standard diet laboratory diet and water (ORC4:Oriental Yeast Co.Ltd.,Tokyo,Japan). The closure of Epiphysis and normal pelvis were confirmed by radiography. Healthy subjects were considered to have closed epiphysis and the femoral had no changes in density or sclerosis and other symptoms of osteonecrosis in radiographs. To make sure that 3 rabbits in each group also underwent MRI. All the processes were performed at Shaheed Rajaei Cardiovascular Medical and Research Center, Tehran, Iran.

Femoral head avascular necrosis induction. Femoral head Osteonecrosis was inducted by surgical dislocation and ligation of the left hip vessels by considering the method used by a previous study (19). In brief, general anesthesia was induced by ketamine (1 mg/kg, i.v.) and midazolam (0.5 mg/kg, i.v.) under veterinary supervision with an anesthetic technician. The animals received one dose of cephazoline (25mg/kg pre op and 25mg/kg bd for 3 day) before surgery. After prep and draping the animal, lied on lateral position, a 4 cm curve incision was made on anterolateral area of the left hip. The fasciae between tensor fasciae latae muscle and

gluteus maximus was opened, gluteus maximus muscle was pulled over and the hip capsulotomy was performed. Then, hip teres ligament was cut and the femur head was dislocated to anterior position. The superior vessels of the femur neck were destroyed by cutting the periosteum all around the femoral neck and the femur neck vessels were ligated. Finally, the hip was relocated, the capsule was closed and the fasciae and skin were sutured in separate layers. At the end of surgical process, the reduction of the hip joint was confirmed by radiography.

Treatment procedure. The rabbits were divided into three groups. The first group, contained ten rabbits, was treated with zoledronic acid; the second group (10 rabbits) received zoledronic acid and vitamin E and the 6 rabbits were considered as control. Zoledronic acid (1mg/kg-subcutaneous injection) was injected for both groups at the 1st and the 4th week. In addition, vitamin E (600 mg/kg, per oral) was added to the food of one of the treated groups similar to a previous study (12). The rabbits were assessed for 12 weeks after the surgery. The radiographic evaluation was performed at the end of 6th and 12th week. The extent of femoral head involvement and severity of Femoral head Osteonecrosis was determined based on Ficat classification (20, 21). Because of the small number of cases that we had, we made a modification on the radiographic classification based on the femoral head collapse. We divided all of the cases in two groups. group one is precollapse cases that include stage 1,2a in ficat classification and post collapse cases that includes 2b,3,4 stages in ficat classification. The data based on Ficat calcification were grouped into good (Ficat stage: 0, I and IIa) and poor (Ficat stage: IIb, 3 and 4).

Also, the femoral head collapse was evaluated by radiographic assessment of Epiphyseal Quotient (EQ). The EQ was calculated by dividing the maximum height of the osseous epiphysis of femoral head by the maximum diameter (22).

All the rabbits were sacrificed at the end of 12th week. Then, New Bone Formation (NBF) and Residual Necrotic Bone (RNB) in head of femur were determined and scored by pathologist. According to NBF, the results divided into good (stage or grade 2 and 3) and poor (stage or grade 0 and 1) groups. Also, based on RNB, the results were grouped into good (stage or grade 0 and 1) and poor (stage or grade 2 and 3) groups.

Statistical analysis. The difference based on Ficat classification at the end of the 6th and 12th week and the changes according to RNB and NBF at the end of 12th week was evaluated by chi-square statistical test. The alteration of EQ mean at the end of 6th week and 12th week was evaluated by Paired t-tests. Furthermore, independent t- test and Fisher's test were used as statistical tests for other comparisons. The statistical difference was considered significant when the p-value was less than 0.05. Statistical analyses were performed using SPSS 22.0 software.

Ethics. The study was approved by the Ethics Committee of the Iran University of Medical Sciences (Tehran, Iran).

Results:

We used the rabbit model to examine the therapeutic effect of zoledronic acid ± vitamin E in treatment of Femoral head Osteonecrosis .The radiologic (Ficat and EQ) and pathologic (NBF and RNB) indices were used to evaluate the effect of the therapeutic plan during a 12-week course.

The radiographs of femoral head were used before surgical intervention to demonstrate the closure of epiphysis [Figure 1] and normal pelvis also at the end of 6^{th} and 12^{th} weeks to assess the radiologic indices in all three groups [Figure 2].

The results of zoledronic acid monotherapy and combination therapy with vitamin E on Femoral head Osteonecrosis based on Ficat classification is presented in table 1.

There was no significant statistical difference between two treated groups and the treated groups with control based on Ficat evaluation at the end of the 6^{th} week (p-value >0.05). Also, there was no significant statistical difference neither between monotherapy and combination therapy groups nor between monotherapy and control based on Ficat evaluation at the end of the 12th week (p-value >0.05). However, the results of Ficat (good) was statistically significant between the zoledronic acid plus vitamin E combination therapy group and control at the end of the 12^{th} week (p-value= 0.048) [table1].

There was not any difference between EQ mean at the end of 6^{th} week and 12^{th} week in zoledronic acid monotherapy group [Table 2]. Although the EQ mean of combination therapy group, treated with zoledronic acid and vitamin E, was increased from 2.990 ± 0.280 to 3.030 ± 0.297 at the end of 6^{th} and 12^{th} week respectively, this difference was not statistically significant (p-value>0.05) (Table 2). Also, the EQ mean of control was decreased from 2.635

± 0.227 to 2.440 ± 0.282 at the end of 6^{th} and 12^{th} week respectively, this difference was not statistically significant (p-value>0.05) [Table 2].

The results presented in table 3 reveals that the EQ mean for the rabbits that had poor Ficat in the monotherapy, combination therapy and control groups were respectively 2.850 ± 0.200, 3.085 ± 0.258 and 2.440 ± 0.272 at the end of the 12th week [Table 3]. The independent t-test revealed that there were not significant statistical differences in comparison of all three groups with each other (p-value >0.05).

Regarding the NBF, 70% of the monotherapy, 77.8% of the combination therapy and 33% of the control groups had good NBF (Table 4). The rabbits with good NBF in each group were compared with the other groups. Yet, there was not any significant statistical difference neither between the treated groups and control nor between the two treated groups (p-value > 0.05).

According to RNB, 90% of the monotherapy group, 90% of combination therapy group and 33% of the control groups had good RNB [Table 4]. Also, the rabbits with good RNB in each group were compared with the other groups. Accordingly, the statistical difference was significant between the treated groups and control (p-value = 0.015).

Seventy percent of monotherapy group was poor based on Ficat at the end of 12th week; in addition, 70% and 90% of monotherapy group had good NBF and good RNB, respectively [Table 5]. Also, 44.4% of combination therapy group was poor based on Ficat at the end of 12th week; 77.8% and 90% of the combination therapy group had good NBF and good RNB, respectively [Table 5]. However, 84% of control group was poor based on Ficat at the end of 12th week; good NBF contained 33% of the rabbits in control group; also, good RNB included the same percent [Table 5]. The Fisher's statistical test was used to compare the results presented in table 5. The comparison of the results of good NBF and good RNB with the result of poor Ficat at the end of the 12th week in monotherapy group revealed that the statistical difference was not significant (p-value > 0.05). Likewise, similar comparison of the mentioned

indices presented no significant statistical difference neither in combination therapy group nor in control (p-value > 0.05).The rabbits with concurrent good NBF and good RNB had 2.751 ± 0.287, 3.091 ± 0.296 and 2.380 ± 0.273 EQ mean (at the end of 12^{th} week) in monotherapy, combination therapy and control groups, respectively [Table 6]. Nonetheless, the independent t-test revealed that there was no significant statistical difference in comparing of three groups (p-value > 0.05).

Discussion:

Avascular femoral capital osteonecrosis shows currently an increasing frequency and the list of risk factors taking part in occurrence of nontraumatic osteonecrosis is on progress (23). Selection of therapeutic method is still controversial although surgical treatment of osteonecrosis is the principal method, eventually trying to preserve the shape of the femoral head (23).

There are various suggestions as treatment for osteonecrosis of the femoral head based on the related mechanisms. For instance, Boss et al. suggested medication of inhibitor(s) of vascular endothelial growth factor to slow down osteoneogenesis because this is the prime factor among the mediators recruiting endothelial cell progenitors into the necrotic epiphysis of the vessels-deprived femoral head in rat model (23). Also, experimental studies recommend treatment with bisphosphonate protects the infarcted femoral head from malformation, but it is without bone anabolic effect (13).

In this animal study the effects of combination therapy of zoledronic acid and vitamin E was evaluated in comparison with zoledronic acid monotherapy on surgical–induced femoral head osteonecrosis in the rabbit model.

The evaluation of the results based on Ficat classification revealed that there was no significant statistical difference between the two groups, received the treatment, with control at the end of 6^{th} week ($P > 0.05$); in addition, no significant statistical difference was found between two treated groups at the end of the 12^{th} group (p-value > 0.05). However, the statistical difference was significant between zoledronic acid plus vitamin E combination therapy group and the control according to Ficat (good) at the end of 12^{th} week ($P < 0.05$).

Both group received the treatment presented less progression to the higher stages of Ficat classification in comparison with control group in 12 weeks. This finding suggests that less femoral head destruction might have been occurred. Also, the group under combination therapy of zoledronic acid and vitamin E had less progression to the higher stages of Ficat classification in comparison with group under zoledronic acid monotherapy in 12 weeks. In detail, 55.6% of the rabbits received zoledronic acid and vitamin E, 30% of the rabbits were under zoledronic acid monotherapy and 16% of the control group were staged good by Ficat classification and the difference between combination therapy group with the control was statistically significant [Table 1] ($P < 0.05$).

There were no significant statistical differences between the groups, received treatment, based on the radiographic-EQ at the end of 6^{th} and 12^{th} weeks ($P > 0.05$). The mean of EQ from the 6^{th} week to the end of the 12^{th} week changed from 2.990 ± 0.280 to 3.030 ± 0.297 in combination therapy group and from 2.635 ± 0.227 to 2.440 ± 0.282 in control. There was no change for the EQ mean of zoledronic acid monotherapy group (2.811 ± 0.278) at the same time course [Table 2]. Although the EQ mean differences were not statistically significant, more decrease of EQ mean in control group in contrast to the treated groups may indicate that the treatment might impede the femoral head collapse.

The pathology evaluation and NBF assessment revealed no significant statistical difference among the three groups (P> 0.05). Nevertheless, a high percentage of the rabbit received treatment (70% of the monotherapy group and 77.8% of the combination therapy) were put in good NBF stage but the less percentage of control group (33%) were in good NBF stage [Table 4]. These results can point to the effects of treatment and it could be confirmed by increasing the sample size.

Based on RNB evaluation, 90% of the both treated group and 33% of control group were in good level [Table 4]; the differences between both treated groups and the control were statistically significant (P<0.05). This confirms the effect of treatment on decreasing femoral head necrosis. But, there was not a significant difference between the monotherapy with combination therapy groups neither numerically nor statistically (p-value >0.05).

As there is significant statistical difference between combination therapy group with the control based on Ficat classification at the end of 12^{th} week; besides, comparing of the both treated groups with the control based on RNB is statistically significant, it seems that treatment with zoledronic acid with/without vitamin E can be effective for Femoral head Osteonecrosis.

Moreover, because the result with significant statistical difference according to Ficat classification was obtained at the end of a 12-week period, it seems that the time course of treatment may be another factor, which can influence the effect of zoledronic acid plus vitamin E therapy for Femoral head Osteonecrosis.

Even though no significant statistical difference was achieved in comparing the results of monotherapy with combination therapy groups, it seems that accompanying of vitamin E may relatively improve the zoledronic acid therapy.

However, according to the findings of this study, the comparison of the results of radiologic assessments with the pathologic findings in three groups was not statistically significant [Table 5 and Table 6].

In accord with our results, a ten-year prospective observational study demonstrated that 3-year-long oral alendronate therapy was a useful choice to delay the requirement for arthroplasty in the young and active patients (14). In this regard, another study by Agarwala et al. showed an improvement in the clinical function, a decline in the rate of collapse and reducing in demand for total hip replacement (15). Also, they indicated that improvement is principally marked if the treatment is initiated before collapse (15).

Besides, Kuribayashi et al. suggested another approach for the prevention of corticosteroid-induced osteonecrosis by giving vitamin E to rabbits and their findings was in agreement with the results of this study, which we used vitamin E in combination with zoledronic acid (12). Although the combination therapy containing vitamin E in comparison with zoledronic acid monotherapy was not statistically significant in this study, the numerical rise of EQ mean at the end of 12^{th} week and NBF (good) [Table 2 and Table 4] may suggest that increasing the sample size can improve the statistical significance. As mentioned above about another trial, it was shown that diet with Vitamin E supplement decreased the incidence of osteonecrosis with statistical significant (12).

Conclusions:

This study demonstrated that there was a significant statistical difference between zoledronic acid plus vitamin E combination therapy group with the control according to Ficat at the end 12^{th} week. Furthermore, RNB presented a significant statistical difference between each treated group with the control. Thus, it can be concluded that both therapeutic regimes including zoledronic acid plus vitamin E as well as zoledronic acid monotherapy were effective in

treatment of Femoral head Osteonecrosis based on one of the pathology indices. However, the Ficat radiologic index was in favor of zoledronic acid plus vitamin E combination therapy. Besides, comparison of the combination therapy with the monotherapy revealed that vitamin E had beneficial effect in treatment although the statistical difference was not significant. Certainly, the animal studies with increased sample size will augment the results and lead to plan the related clinical trials.

Disclosure:

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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