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RESEARCH ARTICLE

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Effectiveness of Injectable Alendronate for Bone Defect due to Osteoporosis

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ABSTRACT

Alendronate is a drug of the bisphosphonate group used for the treatment and prevention of osteoporosis in postmenopausal women. However, when given orally, alendronate can cause indigestion and osteonecrosis of the jaw. It also has a poor bioavailability. Taking these disadvantages into account, an injection formulation of alendronate was created in this study to act on the site locally. Beside alendronate, the injection also contains bovine hydroxyapatite and gelatin as alendronate carriers. Both, besides being able to act as carriers, are also able to reduce bone damage caused by osteoporosis. The aim of this study was to determine the effectiveness of alendronate injection for fractures caused by osteoporosis in mouse models that were ovariectomized. The parameters used in this study were ALP concentration in blood and bone radiology. The results of ALP concentration showed that there were no significant differences in each group. The average ALP concentration of the negative control group was 277.67 ± 46.090, in the positive control group 270.33 ± 189.716, in the BHA-Gel group 406.33 ± 212.547 and in the BHA-Gel Alendronate group 325.00 ± 73.750. Bone radiology results and macroscopic observations still showed bone defects in each group.Yet in the negative control group, the BHA-Gel-Alendronate group and the BHA-Gel group, bone defects were almost entirely ameliorated. On the contrary, bone defects were still present in the positive control group. Based on the results obtained, it was shown that the injection of alendronate has not been shown to significantly overcome osteoporosis fractures.

Keywords: Osteoporosis, Alendronate, Ovariectomy, Bovine hydroxyapatite, Gelatin

INTRODUCTION

Background

Osteoporosis is a bone disorder characterized by decreased bone density, impaired bone architecture and decreased bone strength resulting in fractures(1). Osteoporosis occurs when individuals experience bone mass loss during the aging process and can be caused by many reasons. It is estimated that losing 10% of bone mass in the spine can double the risk of vertebral fracture, while losing 10% of bone mass in the hip may increase a risk of hip fracture by 2.5 times(2). A recent study from the International Osteoporosis Foundation (IOF) revealed that 1 in 4 women in Indonesia within the age range of 50-80 years are at risk of osteoporosis. According to WHO, there are 200 million people in the world suffering from osteoporosis. By 2050, it is estimated that hip fracture rates will increase threefold in men and double in women. The WHO study also revealed that 50% of fractures are upper thigh fractures that can cause lifelong disability and even death. The bone density of European (Caucasian) and Asian race is lower when compared to African race, therefore osteoporosis can easily develop in Asians. This difference in bone density is caused by ethnic diversity as well as by differences in the body's biological systems, such as the susceptibility of genes to the environment or hormonal factors, one of which is the hormone estrogen. The results of the white paper study together with the WHO association in Indonesia in 2007 reported that the prpotion of osteoporosis sufferers above the age of 50 years lie as many as 32.3% in women and 28.8% in men. The Hospital Information System data shows that the incidence of upper thigh fractures caused by osteoporosis is around 200 to 100,000 cases at the age of 40(3).

The weakness of the bisphosphonate group is low bioavailability and its side effects of stomach irritation and osteonecrosis in the jaw. Therefore, an injection formulation was created to be administered locally to the site of bone defects due to osteoporosis. This preparation is biodegradable and biocompatible with a matrix composition consisting of Bovine Hydroxyapatite-Gelatin (BHA-Gel), with Alendronate as the active ingredient. The purpose of making a local injection was to create a dosage form which is able to act locally in order to avoid the side effects of Alendronate. Moreover, the structure of BHA-Gel is also similar to bone structure, thus being able to unite and form new bone to ameliorate bone fractures due to osteoporosis as well as acting as the carrier in the preparation[(4)]. The dosage of Alendronate on the dosage is 0.045 mg. This study aimed to examine the effectiveness of Alendronate injection preparation to treat bone defects due to osteoporosis. The osteoporosis model used was ovariectomized mice from which serum markers alkaline phosphatase was monitored as a marker of bone formation. In addition, bone radiology with X-rays and macroscopic examination were also performed[(5)].

METHODS

The material preparation of the injectable Bone substitute

The preparation in this study weighed 25 mg for 1 mouse, with a BHA: Gelatin: HPMC ratio of 10: 1: 1 and Alendronate weighing 10% of the whole preparation weight. BHA: Gelatin: HPMC was given to 2 groups, the BHA-Gel group and the BHA-Gel-Alendronate group, thus 12 samples were prepared. Alendronate was given in 1 group, so 6 samples were prepared.

Table 1. Amount of material weighed for injectable bone substitute preparations

	Material	Weight of 1 preparation (mg)	Weight of 12 preparations (mg)	Weight of 6 preparations (mg)
1.	BHA	20.8	249.6	-
2.	Gelatin	2.08	24.96	-
3.	HPMC	2.08	24.96	-
4.	Alendronate	0.045	-	0.27
	Total	25	299.52	0.27
Total			299.79	

The Preparation Procedure for Making Injectable Bone Substitutes

The ingredients according to Table 1 were weighed. Gelatin and HPMC were dissolved in destillated water and then heated at 40ºC, while being stirred until homogeneous. Alendronate and BHA were mixed in the mortar, stirred *ad homogen*. Afterwards, the Gelatin-HPMC solution was poured into the mortar slowly while being stirred until a paste was formed. The preparation was then transferred into a watch glass with a parchment paper on top and dried using an autoclave at 40ºC.

The Administration of the Preparation into the Rat's Femur

Before the operation, dried preparations were first dissolved in Na_2HPO_4 2.5% with a ratio of 1: 1. Then, the preparations were put into a 0.1 ml injection syringe. A dose of 25 mg must be injected into the mouse femur by 0.01 ml in injection syringe.

The thighs were sliced with approximately 1.5 cm of length, and then clamps were inserted into the formed cut to locate the bones. After finding the bones, the cut was opened wider using these clamps. The tissue and flesh covering the femur bone were cleaned using tweezers until the bone was visible.

Femur drilling was carried out in all groups of mice with a diameter of 2 mm. Then the preparation was injected in a hole formed on the femur of the mouse. This treatment was carried out at 9 weeks after ovariectomy. Injection was given to only two subgroups of rats while the other two were not given injection preparations.

The cavity was irrigated using normal saline. Suturing was done to close the surgical wound. After the surgery was complete, betadine was applied to the suture area and hypafix was used to close it. Lastly, a 0.2 ml ampicillin injection was given intramuscularly at a dose of 50 mg / 2 ml.

Table 2. Mice Groups and The Treatment

Group	Treatment
1. Negative control	No BHA-Gelatin-Alendronate injection was given
2. Positive control	No BHA-Gelatin-Alendronate injection was given
3. BHA-Gelatin	a BHA-Gelatin injection was given
4. BHA-Gelatin-Alendronate	a BHA-Gelatin-Alendronate injection wa given

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) method. The results of one-way ANOVA statistical analysis obtained a significance level of $p < 0.05$, meaning that there was at least one pair of data being significantly different from the others. A Tukey test was done in order to find out this pair of data. After treatment, the changes in bone defect were observed in each group radiologically and macroscopically.

RESULTS

Table 3. Alkaline phosphatase (ALP) concentration in rat serum

Group	Average alkaline phosphatase concentration (IU/L)
1. Negative control	277.67 ± 46.090 [b]
2. Positive control	467.33 ± 189.716 [b]
3. BHA-Gel	406.33 ± 212.547 [b]
4. BHA-Gel Alendronate	325.00 ± 73.750 [b]

The same superscript in the same column showed no significant difference ($p > 0.05$).

The Effect of Alendronate Injection on Bone Defects Shown in Bone Radiology Imaging

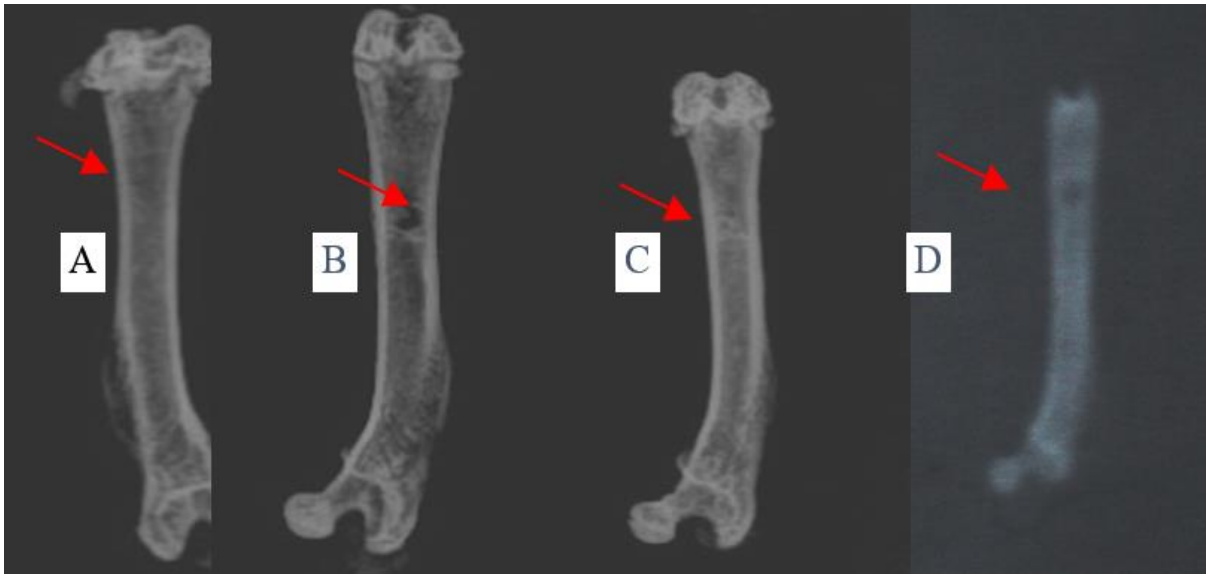

Figure 1. Observation of Rat Femur Bone Radiology: (A) Positive control group, (B): Negative control group, (C): BHA-Gel group, (D): BHA-Gel- Alendronate group. The red arrow points at the fracture present in the bone

Effect of Alendronate Injection on Bone Defects in Macroscopic Observation

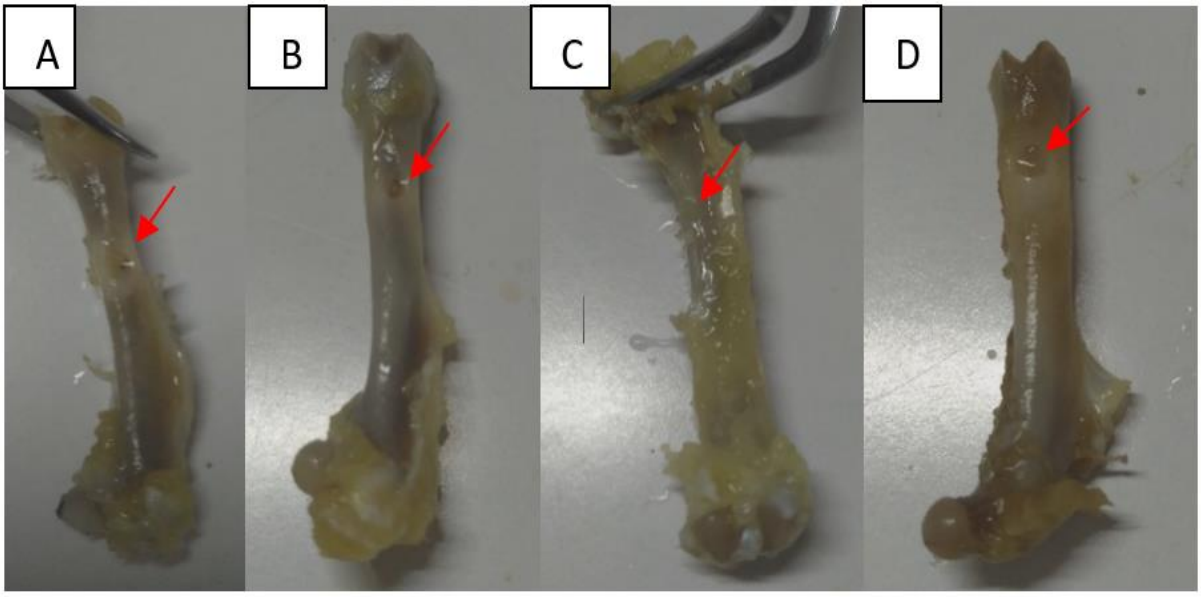

Figure 2. Macroscopic Observation of Rat Femur Bone: (A) Positive control group, (B): Negative control group, (C): BHA-Gel group, (D): BHA-Gel Alendronate group. The red arrow points at the fracture present in the bone

DISCUSSION

The parameter used in this study was alkaline phosphatase, indicating osteoid formation and mineralization in the bone. The bone formation was further observed macroscopically and by radiological X-ray in bone fractures. The average ALP level in positive control group was not significantly different from the negative control group. However, the mean ALP level of the positive control group was higher than the negative group. This leads to the conclusion that there was a decrease in bone mass in the positive control group due to increased osteoclast activity, causing ALP to be released into the bloodstream[(6)].

The injection treatment group BHA-Gel-Alendronate also showed no significant difference in average ALP level compared to the positive control group, even though the average ALP level of the negative control group was lower than the positive control group. A decrease in the average ALP level in group P4 indicated that there was an increase in osteoblast activity in filling the bone fracture that had been resorbed by osteoclasts. Therefore, ALP was bound to bone minerals resulting in its decreased blood level.

The BHA-Gel-Alendronate group and the BHA-Gel group also showed no significant differences. However, the average ALP level of the BHA-Gel Alendronate group was lower than the BHA-Gel group, indicating the effectiveness of alendronate preparations for bone growth because although BHA-Gel is able to fill the bone fracture, alendronate was still necessary to stop the osteoclast activity, so that bone growth process would not remain hampered by osteoclast's activity[(7)].

ALP levels revealed to be insignificant because the injection were prepared to act locally while ALP levels were measured through systemic bloodflow. The ALP levels measured were not only the levels in the preparation but also from other parts of the bone. Therefore, additional data was needed, which was bone radiology data.

Based on observations on the x-ray bone radiology results and macroscopic observations in the positive control group, it was shown that the bone defects in the positive groups were more visible compared to the negative control group, where the fracture was closed. This occured due to decreased bone growth in the positive control group as the result of osteoporosis, while in the negative control group normal bone growth still occured.

The bone defect appeared in BHA-Gel and BHA-Gel-Alendronate groups were still not completely ameliorated, since bisphosphonate drugs reduced osteoblast proliferation and differentiation due to imbalances in bone remodeling, therefore slowing bone growth. The bone fracture in the BHA-Gel group remained since the osteoclast activity is not inhibited by alendronate, thus osteoclasts were still actively resorbing bone[(8)].

CONCLUSION

Injection preparations containing BHA-Gel-Alendronate did not alter the concentration of alkaline phosphatase marker in the blood. From the results of radiological examinations and macroscopic observations, it was shown that injection preparations containing BHA-Gel were able to reduce bone defect due to osteoporosis.

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