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ORIGINAL ARTICLE

Comparison of the prophylactic use of ibandronate and its use in early-stage osteonecrosis in rats with steroid-induced osteonecrosis of the femoral head

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Osteonecrosis of the femoral head (ONFH) can be defined as a condition resulting from the disruption of the intraosseous blood flow of the femoral head due to intrinsic or extrinsic factors caused by both traumatic and non-traumatic reasons. The osteocyte necrosis in the femoral head occurs 2 to 3 h after anoxia and the necrotic area is surrounded by fibrotic repair tissue.[1, 2] New bone formation is usually unable to keep up with the rapid bone resorption resulting from the low mineral density in the femoral head. Due to higher osteoclast activity compared to osteoblast activity, the removal of the dead bone beneath the subchondral bone results in joint collapse over time.

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ABSTRACT

Objectives: The aim of this study was to investigate the effects of ibandronate before and after the onset of osteonecrosis in rats with steroid-induced osteonecrosis of the femoral head.

Materials and methods: A total of 24 female Sprague-Dawley rats were used in this study. Three groups were formed with eight rats in each group. The first group was the prophylactic group that received ibandronate treatment before and after the onset of osteonecrosis (Group PT). The second group received ibandronate treatment three weeks after the development of osteonecrosis (Group TAO). The third group was the control group in which osteonecrosis was created, but only normal saline (NS) was given. At the end of the study, all rats were sacrificed, and their right femoral heads were removed, fixed with formalin, and sent for micro-computed tomography. Hematoxylin-eosin (H&E) and immunohistochemical examinations of the right femoral head sections were performed.

Results: In the PT group, the trabecular thickness was significantly higher compared to those of the TAO and control groups ($p<0.05$). The trabecular thickness did not significantly differ between the TAO and control groups. The trabecular bone pattern factor was significantly higher in the PT group compared to the control and TAO groups ($p<0.05$); however, it showed no significant difference between the TAO and control groups. The incidence of type 2 osteonecrosis in the PT and TAO groups was significantly lower than that in the control group ($p<0.05$). The incidence of tissue-non-specific alkaline phosphatase (TNAPase) immunoreactivity of osteoblast positivity was significantly higher in the PT and TAO groups compared to the control group ($p<0.05$), whereas the incidence of TRAPase immunoreactivity of osteoclastic positivity was significantly lower in the PT and TAO groups compared to the control group ($p<0.05$).

Conclusion: Intravenous administration of ibandronate before the onset of the disease was more effective in the treatment of osteonecrosis in rats with steroid-induced osteonecrosis of the femoral head.

Keywords: Bisphosphonate, femoral head, ibandronate, osteonecrosis, rat.

Many etiological factors such as systemic corticosteroid use, excessive alcohol consumption, hyperlipidemia, smoking, exposure to radiation, use of oral contraceptives, coagulation and hematological disorders, and metabolic diseases have been shown to be considered the non-traumatic causes of ONFH.[1] Among these factors, high-dose corticosteroids for the treatment of autoimmune and other diseases are the most common non-traumatic etiological cause.[3,4] Osteonecrosis of the femoral head is responsible for up to 10% of all total hip replacement surgeries and is the leading reason for total hip replacement in young adults. The results of total hip replacement due to steroid-induced osteonecrosis are worse compared to those of total hip replacement due to osteoarthritis in terms of infection problems, dislocation, osteolysis, and revision.[3,5] Additionally, treating steroid-induced ONFH is quite challenging. In the treatment of ONFH, non-surgical treatment methods such as anticoagulants, bisphosphonates, statins, and teriparatide, which are anabolic agents, have been used. However, these medical treatments are usually used after the development of ONFH.

Bisphosphonates are drugs that bind to bones and inhibit osteoclastic activity, thereby reducing bone resorption.[6-8] They are also considered to be promising agents in preventing early-stage ONFH and femoral head collapse.[9,10] While many animal studies have found the use of bisphosphonates to be effective in the treatment of ONFH, clinical trials have not confirmed these results. Most of the existing animal studies in the literature regarding bisphosphonates are based on ONFH formed with traumatic models. Moreover, there is no study shows the results of bisphosphonate use as a prophylactic treatment in steroid-induced osteonecrosis.

In many autoimmune diseases where high-dose steroids are required, significant osteoporosis can develop due to loss of trabeculae in bone tissue. In individuals using steroids, ONFH may be followed by rapid collapse of the joint cartilage in the femoral head. According to our hypothesis, administering ibandronic acid (ibandronate [IBA]), a commonly used bisphosphonate drug for osteoporosis treatment, in combination with or prior to steroid usage, would treat osteoporosis and reduce the adverse effects associated with potential ONFH. In the present study, we, therefore, aimed to compare the effects of IBA before and after the onset of (early-stage) steroid-induced ONFH in rats through radiological, histopathological, and immunohistochemical evaluations.

MATERIALS AND METHODS

This study included a total of 24 female Sprague-Dawley rats, aged 16 weeks and weighing between 240 to 300 g. All rats were kept under controlled conditions, maintaining a daily temperature of 22°C with a 12-h light/dark cycle. Standardized rodent feed and tap water (ad libitum) were given unlimitedly, regardless of the time before or after drug administration. The rats were followed for seven days in the animal care laboratory before the experiment to exclude the possibility of an underlying disease. Three groups were formed with eight rats in each group. The first group was the prophylactic group that received IBA treatment before and after osteonecrosis (Group PT). The second group received IBA treatment three weeks after the development of osteonecrosis (Group TAO). The third group was the control group in which osteonecrosis was created but only normal saline (NS) was given. The effectiveness of the protocol used to induce osteonecrosis has been proven, and a sham group was not created to maintain a larger number of rats within each group.[3] One week prior to the initiation of osteonecrosis, the PT group was given IBA via the intraperitoneal route at a dose of 15 μg/kg, while the other two groups were given intraperitoneal NS (Table I). In the second week, when osteonecrosis was initiated in all three groups, a second dose of IBA was given to the PT group. In the second week, to induce osteonecrosis in the femoral head,

TABLE I
Agents administered to the study groups

Groups	Week 1	Day 1 of Week 2	Day 2 of Week 2	Day 3 of Week 2	Day 4 of Week 2	Day 5 of Week 2	Weeks 3 and 4	Weeks 5, 6, 7, 8, 9, 10
PT	IBA	IBA+LPS	LPS	MPS	MPS	MPS	IBA	IBA
TAO	NS	LPS	LPS	MPS	MPS	MPS	NS	IBA
Control	NS	LPS	LPS	MPS	MPS	MPS	NS	NS

IBA: Ibandronate; LPS: Lipopolysaccharide; MPS: Methylprednisolone sodium succinate; NS: Normal saline; PT: Prophylactic treatment group (before and after osteonecrosis); TAO: Treatment after osteonecrosis group.

TABLE II
Histological classification of osteonecrosis according to Arlet and Durroux

Type	Description
1	Nonspecific marrow plasmacytosis with few foam cells and focal area of granular eosinophilic necrosis; no bone alteration
2	Marrow necrosis: medullary spaces are replaced by granular eosinophilic necrosis, edema, hemorrhage, fibrous reticulosis, hematopoietic necrosis, and progressive extension of focal lesions
3	Type 2 + trabecular necrosis (50% death of osteocytes)
4	Type 3 + fibrosis and creeping substitution

Arlet J, Durroux R. Diagnostic histologique précoce de l'ostéonécrose aseptique de la tête fémorale par le forage-biopsie. In: La circulation osseuse, compte rendu du ler Symposium International sur la circulation osseuse. Paris: Inserm; 1973. p. 293-302.

20 µg/kg of intraperitoneal lipopolysaccharide (LPS, *Escherichia coli* 055:B5; Sigma-Aldrich, St. Louis, MO, USA) was administered to each group on Days 1 and 2, while 40 mg/kg of intramuscular methylprednisolone sodium succinate injection was given to all three groups on Days 3, 4, and 5. In the third and fourth weeks, Group PT was administered IBA (15 µg/kg, IP), while the other two groups were given NS. Once a week from the fifth week until the end of the study, 15 µg/kg of IBA was administered to the PT and TAO groups, while NS was administered to the control group (Table I). At the end of the study, all rats were sacrificed, and their right femoral heads were removed, fixed with formalin, and sent for micro-computed tomography (micro-CT). Afterward, hematoxylin-eosin (H&E) and immunohistochemical examinations were performed in the Pathology Lab.

Radiological examination

The right femoral heads of the rats were examined at the 3D Medical and Industrial Design Laboratory of Istanbul University (TETLab) for the following parameters: 1- bone mineral density (BMD; g/cm^3), 2- bone volume/total volume (BV/TV; %), 3- trabecular thickness (mm), 4- trabecular number, 5- porosity, 6- trabecular separation (mm), 7- trabecular spacing analysis (1/mm), 8- trabecular bone pattern factor (TBPf; 1/mm), and 9- bone surface area/bone volume (BSA/BV; 1/mm). The trabecular bone pattern factor is a measurement that shows the number of connections in the three-dimensional (3D) trabecular bone and is used to predict trabecular bone loss.

Pathological examination

The femurs of all rats were fixed in a 10% buffered formalin solution for 48 h. After being decalcified in a 10% hydrochloric acid solution for 4 h, the femoral heads and necks were collected in tissue cassettes for macroscopic examination. The samples were processed using alcohol, acetone, xylene, and paraffin in a closed-system tissue tracking device for dehydration. Then, paraffin blocking was performed. 4-µm longitudinal sections were taken and H&E staining was applied to each block. The H&E-stained slides were applied a covering solution and were covered. The histological results were evaluated using the Arlet and Durroux method (Table II).

The 4-µm sections obtained from the paraffin blocks were taken onto slides that were previously coated with poly-L-lysine. The sections were incubated in an oven at 56°C for one night. In the manual immunohistochemical staining, tissue-non-specific alkaline phosphatase (TNAPase [ab 203106] lot GR3181509-5 Rb pab to Alkaline Phosphatase Non-specific; Abcam Inc., Cambridge, UK) was applied with a 1/500 citrate dilution, and tartrate-resistant acid phosphatase (TRAPase [ab 185716] lot GR3275703-5 Rb pab to Tartrate-Resistant Acid Phosphatase; Abcam Inc., Cambridge, UK) was applied with a 1/200 ethylenediaminetetraacetic acid (EDTA) dilution. The rat kidney and liver tissues were collected as a control block and used as a single paraffin block. The Olympus BX51 light microscope (Olympus, Tokyo, Japan) was used in the examination. In the evaluation of TNAPase applied immunohistochemically, the cytoplasmic and membrane staining of osteoblasts at any level was immunoreactively evaluated. In the evaluation of TRAPase applied immunohistochemically, the cytoplasmic staining of osteoclasts at any level was immunoreactively evaluated.

Statistical analysis

Statistical analysis was performed using the SPSS version 26.0 software (IBM Corp., Armonk,

TABLE III
Micro-CT outcomes of the control, PT, and TAO groups

	Control group		Group PT		Group TAO		
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	*p*†
Bone mineral density	0.738±0.037*	0.732	0.673±0.044	0.663	0.708±0.034*	0.692	**0.006**
Bone volume/total volume	32.27±4.14	31.93	36.02±4.60	37.27	34.76±3.80	35.45	0.122
Trabecular thickness	0.700±0.136*	0.681	0.964±0.133	0.944	0.802±0.118*	0.789	**0.004**
Trabecular number	131.9±122.0	80.5	233.0±142.8	216.5	101.4±54.3	98.0	0.100
Porosity	67.73±4.14	68.07	63.97±45.60	62.70	65.22±3.82	64.55	0.122
Trabecular spacing	0.53±0.09	0.52	0.51±0.04	0.51	0.52±0.05	0.52	0.785
Trabecular separation	1.25±0.26*	1.22	1.03±0.18	0.97	1.15±0.19*	1.10	**0.036**
Trabecular bone pattern factor	-10.16±4.76*	-10.71	-3.32±3.67	-2.32	-8.49±2.79*	-8.37	**0.008**
Bone surface area/bone volume	3.22±0.53*	3.03	2.76±0.54	2.55	2.83±0.27	2.80	**0.035**

SD: Standard deviation; PT: Prophylactic treatment group (before and after osteonecrosis); TAO: Treatment after osteonecrosis group; † Kruskal-Wallis (Mann-Whitney U test); * Difference with probhylactic treatment group (before and after avascular necrosis) p<0.05; Significant *p* values are given in bold.

NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max), or number and frequency, where applicable. The distribution of the variables was assessed using the Kolmogorov-Smirnov test. While the comparison of the quantitative data was performed using the Kruskal-Wallis and Mann-Whitney U tests, the comparison of the qualitative data was performed using the chi-square test. A p value of <0.05 was considered statistically significant.

RESULTS

Bone mineral density was significantly lower in the PT group compared to the TAO and control groups (p<0.05). The BMD values between the TAO and control groups showed no significant difference (Table III). The BV/TV ratio did not exhibit any significant difference among all groups. In the PT group, the trabecular thickness was significantly higher compared to those of the

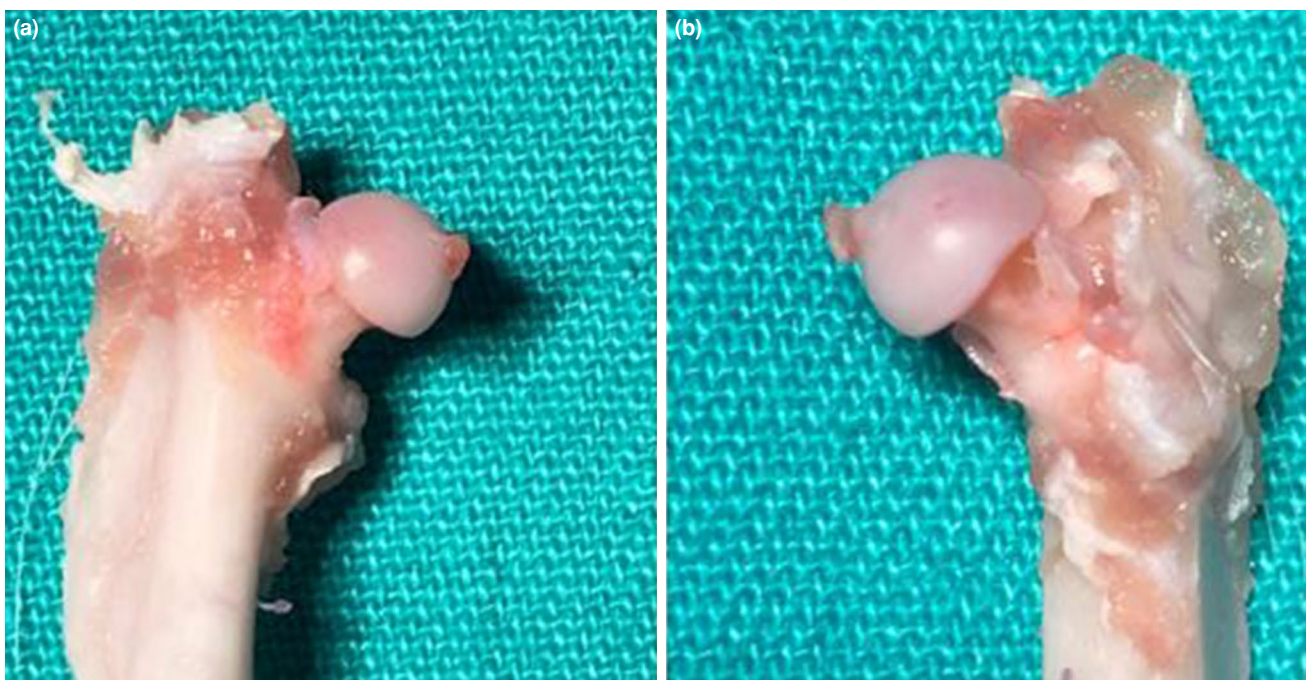

FIGURE 1. (a) Image depicting the right femoral head of a rat from the PT group. No femoral head collapse is seen. **(b)** Image depicting the right femoral head of a rat from the control group. The onset of collapse is seen in the superior femoral head.

FIGURE 2. Horizontal, frontal, and sagittal micro-CT sections from the control, TAO, and PT groups.

TAO and control groups ($p<0.05$). The trabecular thickness did not significantly differ between the TAO and control groups. The trabecular number, porosity, and trabecular separation showed no significant difference among the groups. The trabecular separation was significantly lower in the PT group compared to the TAO and control groups ($p<0.05$), whereas it showed no significant difference between the TAO and control groups. The TBPf was significantly higher in the PT group compared to the control and TAO groups ($p<0.05$); however, it showed no significant difference between the

TABLE IV
Pathological outcomes of the control, PT, and TAO groups in terms of osteonecrosis type, osteoblastic activity, and osteoclastic activity

	Control group		Group PT		Group TAO		
	n	%	n	%	n	%	*p**
Osteonecrosis type							
(+) Positive	0	0.00	8	100.00	8	100.00	0.0001
(++) Positive	8	100.00	0	0.00	0	0.00	
TNAPase immunoreactivity of osteoblasts							
Negative	8	100.00	0	0.00	0	0.00	0.0001
Positive	0	0.00	8	100.00	8	100.00	
TRAPase immunoreactivity of osteoclasts							
Negative	0	0.00	8	100.00	8	100.00	0.0001
Positive	8	100.00	0	0.00	0	0.00	

* Chi-square test. Significant *p* values are given in bold.

TAO and control groups. The BSA/BV ratio was significantly lower in the PT group compared to the control group ($p<0.05$). The BSA/BV in the TAO group did not show a significant difference compared to that of the control group (Table III), (Figures 1 and 2).

The incidence of type 2 osteonecrosis in the PT and TAO groups was significantly lower than that in the control group ($p<0.05$) (Table IV). There was no significant difference between the incidences of type 1 osteonecrosis in the PT and TAO groups. The incidence of TNAPase immunoreactivity of osteoblast positivity was significantly higher in the PT and TAO groups compared to the control group ($p<0.05$). However, there was no significant difference between the PT and TAO groups in terms of TNAPase immunoreactivity of osteoblast positivity. The incidence of TRAPase immunoreactivity of osteoclastic positivity was significantly lower in the PT and TAO groups compared to the control group ($p<0.05$). However, there was no significant difference in the incidence of TRAPase immunoreactivity of osteoclastic positivity between the PT and TAO groups (Figures 3a-d and 4a).

DISCUSSION

In the present study, we investigated steroid-induced ONFH in rats. Our study results showed that the intravenous form of IBA, which is a bisphosphonate drug, showed significant histopathological and

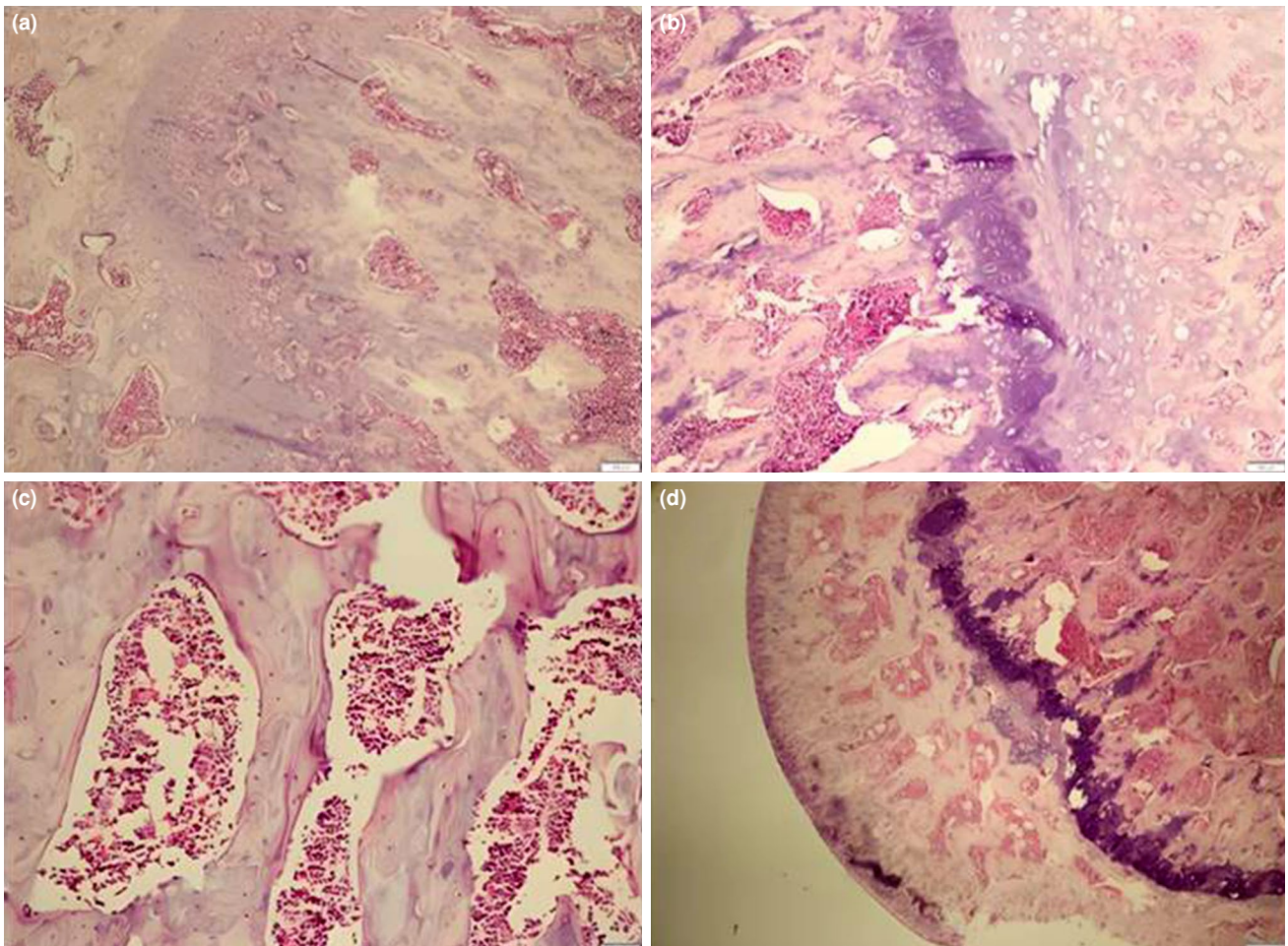

FIGURE 3. (a) Non-specific marrow plasmacytosis with few foam cells and focal area of granular eosinophilic necrosis; no bone alteration. (Group PT, rat no. 8; H&E, ×100). **(b)** Non-specific marrow plasmacytosis with few foam cells and focal area of granular eosinophilic necrosis; no bone alteration. (Group TAO, rat no. 4; H&E, ×100). **(c)** Non-specific marrow plasmacytosis with few foam cells and focal area of granular eosinophilic necrosis; no bone alteration (Group TAO, rat no. 1; H&E, ×200). **(d)** Marrow necrosis: medullary spaces are replaced by granular eosinophilic necrosis, edema, hemorrhage, fibrous reticulosis, hematopoietic necrosis, and progressive extension of focal lesions. (Control group, rat no. 5; H&E, ×40).

immunohistochemical results when used as a prophylactic treatment, as well as helping to preserve the 3D structure of the bone radiologically.

The micro-CT findings confirmed that the trabecular thickness and trabecular number were higher, the trabecular spacing and trabecular separation were lower, and TBPf, which showed the 3D structure of the trabecular bone and the number of connections, was better in the PT group than the other two groups. We also observed that although IBA used after osteonecrosis in rats (Group TAO) returned significant histopathological and immunohistochemical results, it did not provide significant radiological results in the trabecular bone tissue of the femoral head. This may explain why bisphosphonates did not prevent the subchondral collapse following the onset of osteonecrosis in clinical studies. It is well known that bisphosphonates increase trabecular volume more than cortical volume. However, it has been shown that oral bisphosphonates increase trabecular thickness and trabecular number after one year of treatment in clinical studies.[11,12] It takes time for adequate trabecular number and trabecular thickness to be reached in osteoporotic bone with full inhibition of osteoclast activity. Previous studies have shown that suppression of bone resorption begins three months after the initiation of oral bisphosphonate administration. Intravenous formulations of bisphosphonates have been shown to have a more rapid effect, suppressing bone resorption one week after administration.[13,14] Saag et al.[13] demonstrated that N-terminal telopeptide, which is commonly used to assess the bone turnover rate, was detected in the urine one week after the intravenous administration of zoledronate, while it was detected only three months after the use of oral bisphosphonates. This indicates that intravenous bisphosphonates exert their effects more rapidly than oral bisphosphonates. In our study, we used the intravenous form of IBA. Nevertheless, there may not be enough time to prevent the rapid collapse of the femoral head in patients with ONFH. Furthermore, it is not clear whether the bisphosphonates reach the osteonecrotic part of the bone in sufficient doses. A study has shown that the distribution of bisphosphonates is less in the osteonecrotic femoral head compared to the revascularized femoral head.[15] Indeed, our study attempted to investigate the effect of bisphosphonates on bone architecture when they were given prophylactically before the development of ONFH. In patients who will receive high-dose steroids (such as in the treatment of lymphoma or multiple sclerosis), starting with the intravenous form of bisphosphonates, which may show a more rapid effect, may preserve the trabecular bone architecture. Thus, it can prevent the subchondral collapse of osteonecrosis in the joint and subsequent complications such as osteoarthritis. Additionally, the risk of osteonecrosis cannot be ignored in patients who receive high-dose steroids. A study reported that the rate of high-dose steroid use in acute lymphoblastic leukemia (ALL) ranged from 2.3 to 7.7%.[16]

In our study, using the intravenous form of IBA reduced the severity of osteonecrosis histopathologically both in the PT and TAO groups according to the Arlet and Durroux classification. In both the PT and TAO groups, type 1 osteonecrosis was predominant, while in the control group, type 2 osteonecrosis was observed. Non-specific marrow plasmacytosis with few foam cells and a focal area of granular eosinophilic necrosis were observed in the PT and TAO groups (Figures 3a-c). In the control group, medullary spaces were replaced by granular eosinophilic necrosis, edema, hemorrhage, fibrous reticulosis, hematopoietic necrosis, and progressive extension of the focal lesion (Figures 3d and 4a). While no bone alterations were observed in the PT and TAO groups, marrow necrosis was present in the control group. Bisphosphonates may stimulate osteoblast differentiation and prevent osteoblast apoptosis.[17,18] This effect of bisphosphonates can reduce the degree of osteonecrosis histopathologically, as in our study.

In the current study, we also evaluated osteoblastic and osteoclastic activities immunohistochemically. Treatment with IBA in the PT and TAO groups sustained osteoblastic activity but suppressed osteoclastic activity, whereas the control group exhibited the opposite pattern (Figures 4b-d). Our findings are consistent with previous studies indicating that inhibiting osteoclastic activity leads to an increase in osteoblastic activity.[6,10] Some authors have reported that osteoclasts become more active and develop a longer lifespan in the presence of osteonecrosis.[19,20] Bisphosphonates suppress the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase pathway in cholesterol synthesis and inhibit the farnesyl pyrophosphate synthase activity within osteoclasts, both of which accelerate osteoclast death.[21,22]

Bisphosphonates are widely used in many diseases, including osteoporosis, malignancies that metastasize to bone, multiple myeloma, and Paget's

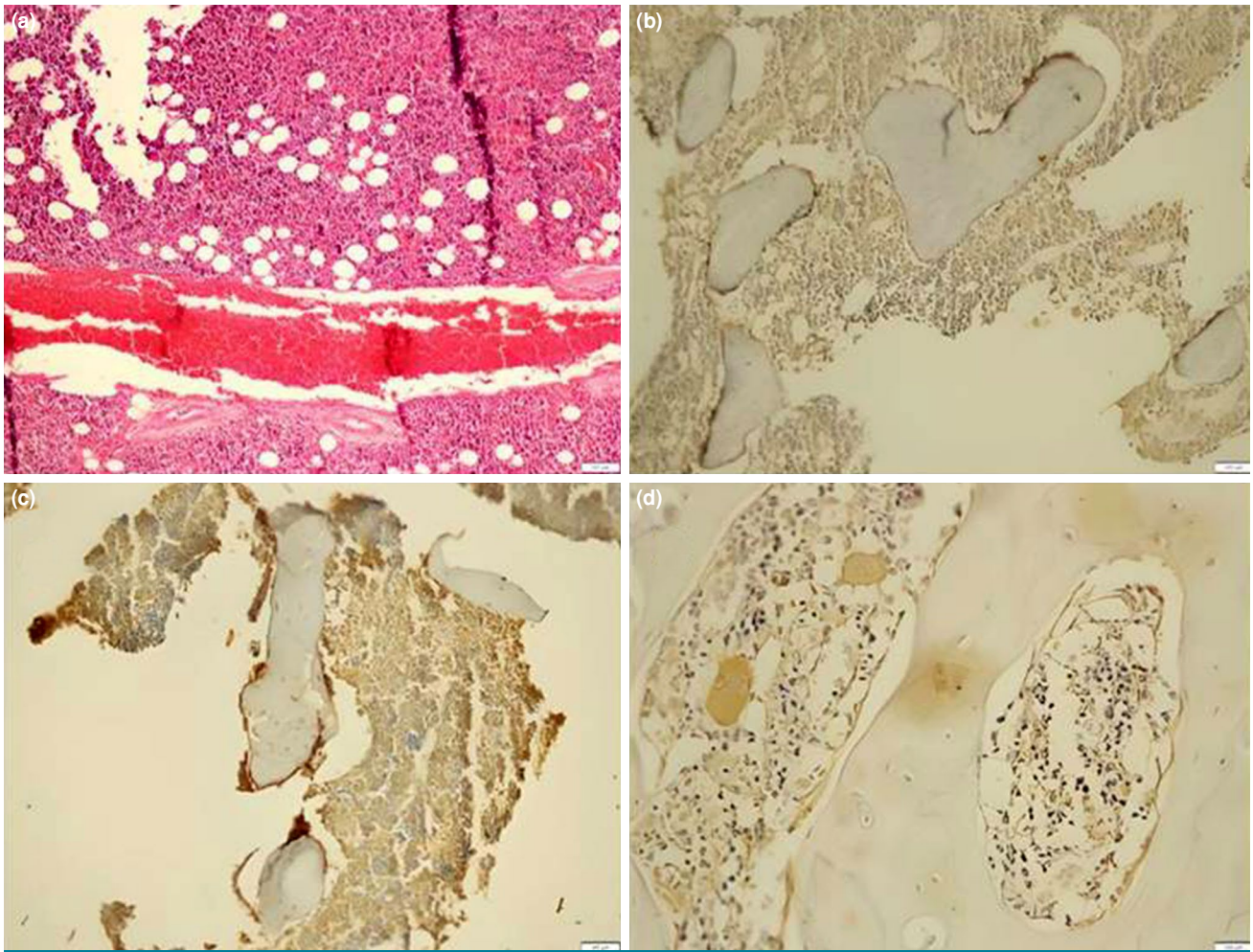

FIGURE 4. (a) Marrow necrosis: medullary spaces are replaced by granular eosinophilic necrosis, edema, hemorrhage, fibrous reticulosis, hematopoietic necrosis, and progressive extension of focal lesions (Control group, rat no. 6; H&E, ×100). **(b)** TNAPase positivity of osteoblast (Group PT, rat no. 1; TNAPase, ×200). **(c)** TNAPase positivity of osteoblast (Group TAO, rat no. 4; TNAPase, ×200). **(d)** TRAPase positivity of osteoclast (Control group, rat no. 2; TRAPase, ×400).

disease, which are caused by osteoclasts and result in bone loss.[23-25] These drugs have also been used in the treatment of ONFH. Despite several animal[6,10] and clinical studies[9,26] showing the efficacy of bisphosphonates in the treatment of ONFH, some authors have reported controversial findings. In their clinical studies, Lee et al.[27] used zoledronate to treat patients with Steinberg Stage I and II ONFH with moderate and large necrotic areas. However, the authors showed that zoledronate was not successful in preventing femoral head collapse or reducing the need for total hip arthroplasty. In another study, Chen et al.[28] utilized alendronate to prevent femoral head collapse; however, they observed that the treatment did not reduce the progression of the disease or improve the quality of life. However, there are also opposing views in the literature. In a clinical study involving 60 patients with ONFH, Agarwala et al.[29] demonstrated that the use of oral bisphosphonates prevented the progression of the disease, reduced femoral head collapse, and improved clinical outcomes. In another study of the aforementioned authors, they administered a combination treatment of intravenous zoledronate and weekly oral alendronate of 70 mg to 31 patients with adolescent ONFH who developed the condition during or after high-dose dexamethasone treatment for ALL.[30] In their follow-up of an average duration of 50.35 months involving 34 hips, the authors reported satisfactory results and the need for total

hip arthroplasty was 0%, 5%, and 22.5% for Ficat-Arlet Stage I, II, and III osteonecrosis, respectively.

Currently, steroids are widely used in many diseases. In many autoimmune diseases where high doses and long-term steroid use is necessary, serious osteoporosis can occur due to trabecular loss. If ONFH occurs during this steroid use due to existing osteoporosis, the femoral head joint cartilage can quickly collapse. This is precisely the area where we believe bisphosphonates can be effectively used. By combining IBA with steroids or administering it prior to steroid use, we believe that it would effectively address osteoporosis and reduce the potential adverse effects associated with ONFH (Figure 1).

Beckmann et al.[31] reported that the administration of enoxaparin as adjuvant therapy along with steroids reduced ONFH. They argued that this was achieved by the antithrombotic of enoxaparin. There are both animal and clinical studies related to the use of bisphosphonates after the development of osteonecrosis in the literature.[6] In the literature, we could not find a study regarding the use of IBA as an adjuvant therapy combined with steroids to reduce the severity of ONFH.

Cases of jaw osteonecrosis after surgical intervention have been rarely reported in patients using bisphosphonates.[32] However, in a clinical study conducted by Felsenberg et al.[33] in 17,000 patients receiving bisphosphonate treatment, no case of jaw osteonecrosis was reported. Another complication reported to be associated with the long-term use of bisphosphonates for 5 to 10 years is subtrochanteric insufficiency fracture. This fracture formation is attributed to excessive reshaping of the bone, but no biochemical or histological data are provided.[34,35] We believe that it is more reasonable to receive bisphosphonate treatment while using high-dose steroids, but not for a long period. In this way, we believe that complications of this type can be eliminated during the use of bisphosphonates.

The main limitations to the present study are the lack of a sham group and relatively short study period (10 weeks).

In conclusion, our study results showed that the intravenous administration of IBA before the onset of the disease was more effective in the treatment of osteonecrosis in rats with steroid-induced ONFH. The results were evidenced by the preservation of the 3D architecture of the bone and the increase in trabecular thickness observed in micro-CT scans. In addition, the use of IBA reduced the degree of bone edema, inhibited osteoclastic activity, and increased osteoblastic activity. For patients using high doses of steroids, we believe that the intravenous administration of bisphosphonates prior to osteonecrosis development may prevent the rapid collapse of the femoral head and potentially save time for surgical intervention, if needed.

Ethics Committee Approval: The study protocol was approved by the Bağcılar Training and Research Hospital Animal Experiments Local Ethics Committee (date: 06.01.2029, no: HADYEK/2018-39).

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Study design, writing: S.Ç.; Writing, analysis: M.F.D.; Data collection: A.A.; Study design, analysis: A.Ç.; Critised of drafited the article: Y.M.D.; Approved last version, analysis: A.Ç.

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