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Evaluation of Short Term Effects of Hyperbaric Oxygen and Enoxaparin Treatments in Avascular Necrosis of Femoral Head

Femur Başı Avasküler Nekrozunda Hiperbarik Oksijen Tedavisinin ve Enoksaparinin Kısa Dönem Etkilerinin Araştırılması

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Öz

Amaç: Femur başı avasküler nekrozu (AVN), femur başını besleyen damarların hasarlanması veya tıkanması sonucu ortaya çıkan kemik ve kemik iliği nekrozudur. Özellikle gençlerde ve orta yaşlarda görülen, büyük oranda cerrahi müdahale gerektiren bir hastalıktır. Cerrahideki gelişmelere rağmen bu hastalarda önemli oranda kalça protezi uygulaması gerekmektedir. Hiperbarik Oksijen Tedavisi (HBOT) tek başına veya cerrahi tekniklerle beraber femur başı avasküler nekrozunda bazı klinik olgularda başarılı sonuçları bildirilmiş bir tedavi yöntemidir. HBOT damar hasarına veya damar tıkanıklığına bağlı gelişen iskemik hastalıklarda da kullanılmaktadır. Enoksaparin pıhtılaşma sisteminde bulunan faktör Xaantagonistidir. Antikoagülan etkisinin yanında yapılan çalışmalarda kemik doku üzerinde osteopeni oluşturduğu, osteoblast gelişimini engellediği, osteoklast aktivitesini arttırdığı görülmüştür.
Gereç ve Yöntemler: Bu çalışmada sıçanlarda deneysel olarak oluşturulan femur başı AVN'de HBOT ve Enoksaparinin etkinlikleri araştırılmıştır. Bu amaçla 64 sıçanın sol femur başlarına avasküler nekroz modeli uygulandı ve tedavilerin tek başına ve beraber uygulanmasının sonuçları araştırıldı.
Bulgular: Çalışma sonucunda HBOT alan ve Enoksaparin tedavisi alan sıçanlarda yeni kemik yapımının arttığı, remodelizasyon ve kıkırdak değişikliklerinin kontrol grubuna göre daha az olduğu görülmüştür. Nekrotik dokuların temizlenme hızının HBOT ile arttığı görülmüştür. HBOT ile Enoksaparinin beraber uygulanmasıyla en iyi sonuçlara ulaşılmıştır.
Sonuç: Bu çalışma HBOT'nin ve Enoksaparin tedavisinin tek başına veya kombine olarak femur başı AVN'de olumlu sonuçlar oluşturduğunu göstermektedir ve femur başı AVN hastalığı tedavisinde yapılan klinik çalışmaları desteklemektedir.

Anahtar Kelimeler: Hiperbarik oksijen tedavisi, avasküler nekroz, enoksaparin

Abstract

Aim: Avascular necrosis of the femoral head (AVN) is the necrosis of the bone and the bone marrow resulting from the injury or the occlusion of the blood supplying vessels of the femoral head. Specially it is seen in mostly young and middle aged individuals and requiring mostly surgical procedures. Despite all advances in the surgical procedures, still a high rate of the hip prosthesis is a requirement in the treatment. Hyperbaric Oxygen Therapy (HBOT) alone or in combination with surgery is reported to be effective in some clinical AVN cases. HBOT is also a treatment method which is used in ischemic diseases resulting from vascular injuries or occlusions. Enoxaparin is an antagonist of factor Xa which is found in the coagulation system. Beside its anticoagulant effect it has been shown that it has an osteopenic effect, inhibits osteoblast maturation and increases osteoclast activity in the bone.
Material and Methods: In this study effects of enoxaparin and HBOT were investigated in the treatment of experimentally formed AVN of the femoral heads of the rats. For this purpose, avascular necrosis model was applied to the left femoral heads of 64 rats and the results of the treatments alone and together were investigated.
Results: In this study results have shown that an increase in the new bone formation, and remodelisation with less changes in the cartilage tissue than in the controls. An increase in the necrotic tissue clearing rate observed in the HBOT applied groups. The best results were achieved with the HBOT and enoxaparin combined applications.
Conclusion: In this study HBOT alone, enoxaparin alone or combination of these two treatments have a positive effect in the experimental femoral head AVN and supporting the clinical studies in the femoral head AVN.

Key words: Hyperbaric oxygen therapy, avascular necrosis, enoxaparin

INTRODUCTION

Avascular necrosis of the Femur Head (AVN), is a bone and bone marrow necrosis occurring due to the disruption of epiphyseal supply. Although it develops depending on many etiological factors, same clinical symptoms and pathophysiologic course occur. Despite the medical and surgical treatments, a considerable part of patients (10-20%) requires total hip prosthesis application after the development of avascular necrosis of the femur head. Early diagnosis in these patients delays total hip prosthesis implementation. Given that the average life of total hip

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prosthesis is 12 years; the burden on social security system brought by repeating surgical interventions, rehabilitation applications and prosthesis costs is remarkable (1).

Surgical treatment is usually performed in the treatment of avascular necrosis of the femur head due to delayed diagnosis. Non-surgical treatment can be administered alone or in combination with surgical methods in the cases with early diagnosis. Hyperbaric Oxygen Therapy (HBOT) is a method which efficiency is being studied, and used in early stage avascular necrosis. The amount of oxygen dissolved in the plasma independently from hemoglobin is increased with oxygen inhalation at high pressures, providing more oxygen delivery to tissues. HBOT is considered to be effective in the treatment of AVN, owing to preventing hypoxia, antiedema effect, increasing diffusion distance in tissues, and increasing osteoblastic and osteoclastic activities (2). Enoxaparin functions as Factor Xa inhibitor found in the mechanism of coagulation. Enoxaparin is being studied as on of the non-surgical treatment methods in avascular necrosis of the femur head (2). The objective of this study was to investigate effectiveness of HBOT and enoxaparin therapy, individually or in combination, in avascular necrosis of the femur head induced experimentally. No study found in the literature, using HBOT and enoxaparin together.

MATERIAL AND METHODS

This study was approved by the Istanbul University Experimental Animals Ethics Committee (Istanbul University, 2009/126). A total of 64 Wistar albino female rats, weighing 250-260 g were used in this study. Study groups, appropriate surgical method and treatments are given in Table 1. At the end of the experiment, the rats were sacrificed using high-dose anesthetic agent.

Experimental AVM model developed by Norman et al. was applied on the rats (3). The rats were anesthetized with intraperitoneal (IP) Ketamine (Ketalar; 50 mg/Kg) and Xylisin (Rompun; 5 mg/Kg) injection. The rats were laid on the right side on sterile covers on which the surgical procedure will be performed. An incision was made via the left trochanter major, and hip joint was reached after gluteus maximus and gluteus medius fibers were cut. The teres ligamentum was cut, the hip was dislocated, rugination of the femoral neck, the intertrochanteric region was scratched and damaged. After the procedures were completed, the hip was reduced and its layers were closed. Subcutaneous and cutaneous sites were sutured (Figure 1, Figure 2).

Table 1. Study groups and treatment methods

Group	Application	Animal Number
1	Control 15 days	8
2	HBOT 15 days	8
3	Enoxaparin 15 days	8
4	HBOT+ Enoxaparin 15 days	8
5	Control 30 days	8
6	HBOT 30 days	8
7	Enoxaparin 30 days	8
8	HBOT+ Enoxaparin 30 days	8

After the animals were placed in the experimental pressure room, ventilation was made with 100% oxygen for 10 minutes, and a pressure of 2.5 ATA was reached. The treatments were administered with a duration of 90 minutes and a pressure of 2.5 ATA. After the surgical procedure, and waiting for anesthetic effect to end, the animals were taken to the HBO therapy within the first 4 hours. The treatment was applied in the animals once a day. HBO therapy was administered for 15 days in two groups, and 30 days in the other two groups. Enoxaparin (Clexane) treatment was initiated on the same day after the surgical procedure. The animals received 1 mg/Kg/day dose as IM based on the method described by Norman et al. (4). Prepared injectors of 0.6 cc containing 60 mg enoxaparin (Clexane 0.6 cc) were used in the study. Clexane of 0.1 cc was diluted in 10 cc 0.9% NaCl. Enoxaparin treatment was applied for 15 days in two groups, and 30 days in two groups.

Femur bones removed from the sacrificed rats were fixed in 10% buffered formalin for one week. After the fixation, The pieces taken from the femoral head to the diaphysis extension from the pieces decalcified

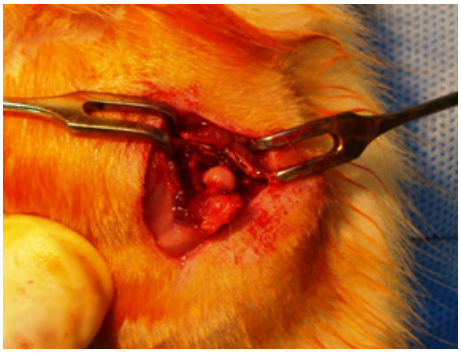

Figure 1. Exposure of the femoral head from the articular capsule by cutting the Lig. Teres

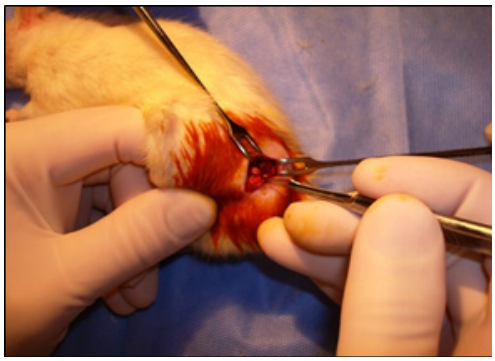

Figure 2. Traumatization of the femoral neck with a scalpel

in the solution, which prepared with 1/1 ratio of 50% formic acid and 20% sodium citrate solutions, were subjected to routine treatment. Section of 5-7 micron cut from the prepared paraffin blocks were stained with hematoxylin eosin and examined under the light microscope. In the histopathologic examination, necrosis, new bone formation, inflammatory reaction, and fibrosis area were studied in each femoral head, and percentile rates were obtained; while remodalization and cartilage changes were scored with a staging between 0-3 (Tables 2-3).

Data obtained from the study were statistically analyzed using SPSS Windows 15.0 software. The results were statistically evaluated in the first 4 group (followed up for 15 days) and the last 4 groups (followed up for 30 days) in themselves. Necrosis formation, new bone formation, inflammatory reaction, and fibrosis results expressed with percentages were evaluated with Kruskal-Wallis One Way Variance Analysis. Binary comparisons for the parameters creating significant difference were evaluated with Bonferroni corrected Mann-Whitney U test. In evaluation of the results of cartilage changes and remodeling, categorical values were analyzed using Fisher exact test. In addition, binary comparisons between Groups 1 and 5, Groups 2 and 4, Groups 3 and 5i and Groups 4 and 8 that were followed up with similar treatments were evaluated with Mann-Whitney U test. The results were evaluated at $p<0.05$ significance level at 95% confidence interval.

Table 2. Remodelization pathology scores patoloji skorlaması

Remodelization	Scores
No change	0
No significant change in the femporal head	1
There is deformation in the femoral head, but the spheric shape is presevred	2
Significant deformation	3

Table 3. Cartilage degeneration pathology scoring

Cartilage Degeneration	Scores
No change	0
Matrix disruption and basophilia loss	1
Thinning + irregular distribution of chondrocytes + thin pannus	2
Fibrosis tissue + thick pannus + splitting	3

RESULTS

The amount of necrotic bone were statistically lower in Group 2 (HBOT) and Group 4 (HBOT+ Enoxaparin) that were followed up for 15 days compared to Group 1 (Control) ($p<0.05$). Although not statistically significant among the groups followed up for 30 days, the amount of necrotic bone was lower in Group 6 (HBOT) and Group 8 (HBOT+ Enoxaparin) compared to Group 5 (Control). Although not statistically significant, the amount of necrotic bone was higher in the groups followed up with Enoxaparin treatment for 15 days and 30 days (Groups 3 and 7) compared to the groups administered HBOT (Groups 2, 4, 6 and 8), and lower compared to the control groups (Groups 1 and 5). Among the groups followed up for 15 days, new bone formation was statistically higher in Group 2 (HBOT) and Group 4 (HBOT+ Enoxaparin) compared to Group 1 (Control) ($p<0.05$). Among the groups followed up for 30 days, new bone formation was statistically significant in Group 6 (HBOT), Group 7 (Enoxaparin) and Group 8 (HBOT + Enoxaparin) compared to Group 5 (Control) ($p<0.05$). Among the groups followed up for 30 days, the amount of fibrotic tissue was statistically lower in Group 8 (HBOT+ Enoxaparin) compared to the other groups ($p<0.05$).

The amount of fibrotic tissue was the least only in the groups administered a combination of HBOT and Enoxaparin therapies. Whereas there was no statistically significant difference between the groups followed up for 15 days in terms of femoral head remodeling, the best results were obtained in Group 6 (HBOT) and Group 8 (HBOT+ Enoxaparin) among the groups followed up for 30 days. Remodeling was found to give more positive results in HBOT group followed up for 15 days, compared to HBOT group followed up for 30 days. In the evaluation of articular

Table 4. p values of the groups followed up for 15 days

	Necrosis Median (Min-Max)	p	New Bone Formation Median (Min-Max)	p	Inflammatory Reaction Median (Min-Max)	p	Fibrosis Median (Min-Max)	P
Group 1	60,00 (40-80)	0,002	11,00 (8-40)	0,004	16,50 (8-75)	0,036	27,50 (8-55)	0,676
Group 2	13,50 (8-45)		50,0 (15-65)		12,00 (0-15)		15,00 (0-50)	
Group 3	28,50 (0-80)		11,0 (8-75)		11,00 (8-20)		37,50 (10-60)	
Group 4	4,50 (0-15)		55,0 (12-75)		6,50 (0-15)		12,00 (0-15)	

cartilage changes, among the groups followed up for 30 days, statistically better results were obtained in Group 6 (HBOT) and Group 8 (HBOT+ Enoxaparin) ($p<0.05$). Inflammatory reaction was lower in all groups followed up for 30 days (Groups 5, 6, 7, and 8) compared to the groups followed up for 15 days (Groups 1, 2, 3 and 4) ($p<0.05$). Although not statistically significant, less inflammatory reaction was observed in the groups administered HBOT and Enoxaparin therapy compared to the control groups (Tables 4-5).

DISCUSSION

Avascular necrosis of the femur head is a disease, which mechanism of occurrence and treatment remain controversial. It is most commonly seen in 3rg and 4th decades of life, and mostly causes progressive destruction in the femoral head. Despite many medical and surgical methods are performed, satisfactory results can not be obtained. Collapse is seen in hip joint of a large portion of patients, and total hip arthroplasty is needed. Total hip arthroplasty operations decrease patients' quality of life, and expose them with a costly and long treatment process; because this operation is a major surgical intervention, the prostheses have a life about 10 years and require replacement, and the patients encounter with the risk of postoperative infections (1). Manifestation of the signs and diagnosis of the disease take place at later stages. Pain which is the first sign is mostly neglected, facilitating the disease to progress to advanced stages (5). Despite surgical procedures are applied for improvement of the disease at later stages, satisfying results can not be obtained, and form sequelae. Today, detection of AVM at early stages is possible with the advancements in radiologic examination that have become widespread (6). Although physiopathology of the disease develops due to quite variable factors, necrosis formation emerges due to disruption of femoral head supply. Effectiveness of medical therapies in addition to surgical methods or separately is being studied in the eray period of Avm where still no collapse in the femoral head and no permanent changes in articular cartilage. HBO therapy provides more oxygen transport to the necrotic area, increasing osteoblastic and osteoclastic activities, decreasing edema, and helps to meet oxygen demand which is increased in this region (1, 7). Studies have shown that HBOT accelerates wound healing, decreases inflammation, and increases neovascularization (8). Recent studies on the etiology of AVN have found that majority of AVN patients in whom the cause is not clear and are classified as idiopathic, the disease is resulted from blood coagulation system disorders (9). Enoxaparin is a pharmacological agent which belongs to low molecular weight heparin groups and is used in hypercoagulability diseases or in patients with the risk of thrombosis (10). Therapeutic effect of Enoxaparin in AVM of the femur head is being studied in recent years (2). In the literature screening, we found no

Table 5. p values of the groups followed up for 30 days

	Necrosis Median (Min-Max)	p	New Bone Formation Median (Min-Max)	p	Inflammatory Reaction Median (Min-Max)	p	Fibrosis Median (Min-Max)	P
Group 5	2,50 (0-45)	0,068	11,00 (5-45)	0,000	10,00 (0-50)	0,008	50,00 (0-85)	0,000
Group 6	0,00 (0-0)		77,50 (45-85)		0,00 (0-0)		28,50 (10-50)	
Group 7	0,00 (0-12)		47,50 (40-85)		0,00 (0-12)		10,00 (8-45)	
Group 8	0,00 (0-5)		77,50 (45-85)		0,00 (0-0)		0,00 (0-8)	

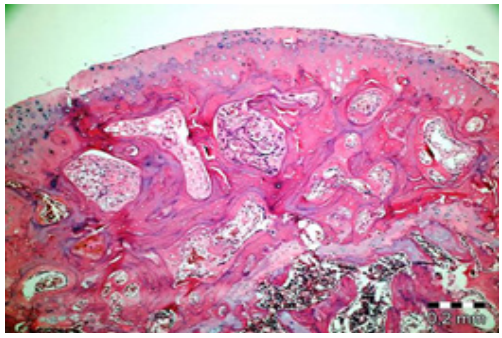

Figure 3. 30 days control group: Irregular epiphysis cartilage (1) new bone trabeculae separated from the bony tissue with apposition lines on the cartilage (2) and partly fibrotic bone marrow involving necrotic isles between them (3)

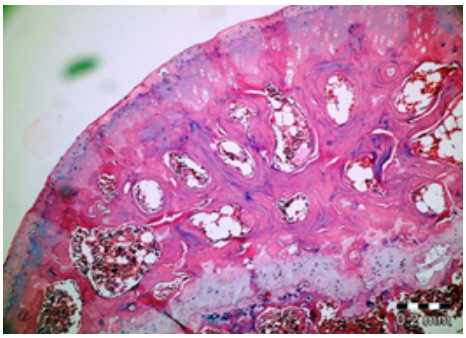

Figure 5. 30 days enoxaparin group: Necrotic bone trabeculae on the epiphysis cartilage with preserved thickness, but irregularly arranged chondrocytes (1), surrounding appositional new bone formation and partly fibrotic bone marrow between them (2)

experimental study using HBOT and Enoxaparin simultaneously. HBO therapy is known to make many pharmacological agents potentialized in its area of usage (11). In this study, we investigated individual and combined treatments of HBOT and Enoxaparin.

In a study of animal model developed by Norman et al., it was demonstrated that bone marrow edema developed in the 2nd day, pyknotic nucleus osteocytes formed from the 5th day, or empty bone lacunas were seen and necrosis was formed. It was reported that, because of the rapid breakdown and formation in rodents, necrosis began to be eliminated in animals sacrificed at the 42th day, and therefore treatments lasting longer than 30 days should not be performed in rodents in experiments (3). In our study, while necrosis was observed in the control and HBO groups followed up for 15 days; bone necrosis was not detected in 2 and 4 rats respectively in the Enoxaparin and HBO+ Enoxaparin groups. Bone necrosis was found in only one rat among the rats in HBO and HBO + Enoxaparin groups followed up for 30 days, while bone necrosis was observed in 4 rats in the control group and 3 rats in the Enoxaparin group. When compared with the study by Norman et al., not disappearing necrosis found in the control group followed up for 15 days showed similar results, while lack of necrosis in 4 rats in the control group followed for 30 days was inconsistent. In our study, necrosis disappeared in an earlier time.

In the same model investigating the effects of HBO therapy, Norman et al. examined the amount of necrosis, osteoneogenesis, remodeling, and articular cartilage changes with histopathological evaluation. No significant difference was found among the groups sacrificed on the 2nd, 7th, and 21th days. It was observed that, among the groups sacrificed on

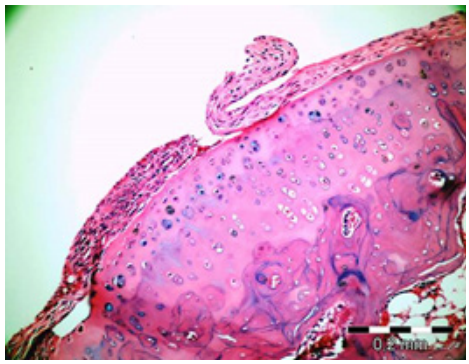

Figure 4. 30 days HBOT group: (1) pannus formation on the surface, necrotic bone trabeculae localized towards the metaphyseal region beneath it (2), fatty bone marrow between them (3)

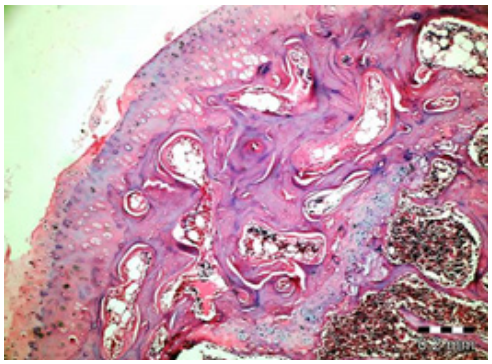

Figure 6. HBOT+ enoxaparin group: Irregular articular cartilage (1), new bone trabeculae (2) and partly cellular bone marrow (3)

the 42th day, appositional and intramembranous new bone formation was higher, remodeling occurred at a higher rate, and necrosis rate was lower in HBO group compared to the control group. No significant difference was found between both groups in terms of degenerative changes and articular cartilage changes (7). In our study, the amount of necrosis was lower and new bone formation was higher in HBOT and HBOT + Enoxaparin groups that were followed up for 15 days. Lack of a significant difference in terms of remodeling and cartilage changes may be attributed to the small number of animals in the groups. It was found that new bone formation was statistically higher, and the amount of fibrotic tissue, remodeling and cartilage changes were lower in HBOT and HBOT + Enoxaparin groups that were followed up for 30 days compared to the control group. No statistically significant difference was found in terms of the amount of necrosis. In our study, HBO treatment alone and in combination with Enoxaparin were more efficient than the study by Norman et al. The most positive results were obtained with the combined treatment among the groups followed up for 15 days and 30 days.

In a study by Barth et al., damage was induced in the bone cortex with drilling application in femoral metaphysis of rats, and the rats were divided into three groups. The first groups was the control group, the second group was treated with HBOT once a day (2 ATA, 100%O_2, 90 minutes), and the third groups was treated with HBOT twice a day (2 ATA, 100%O2, 90 minutes). The rats were sacrificed at the 2, 7, 14, 21, 28, and 35. days, and effects of HBOT administered once or twice again on bone healing were investigated. The rats were evaluated with electron microscopy, light microscopy, and histomorphometry methods. Improvement with endochondral ossification in the control group and the group administered HBOT twice a day, while primary ossification was observed in the group administered HBOT once a day. According to the histomorphometric findings, it was shown that HBOT once a day increased bone repair and vascularization compared to the control group, and HBOT twice a day decelerated bone repair and vascularization compared to the control group (12). In the present study, HBOT therapy was applied once a day with 2.5 ATA, 100%O_2, 90 minutes protocol. Similarly we observed that osteoblastic new bone formation and remodeling were increased in the groups followed up for 15 days and 30 days.

In a study by Jones et al., avascular necrosis was induced in the femoral head of rabbits by performing osteotomy to the femoral heads, and the effects of HBO therapy were investigated. HBO therapy was applied twice a day with 2 ATA, 60 minutes and hyperoxygenation for 3 hour after each session in the first 16 days, and twice a day hyperoxygenation for 3 days in the subsequent 12 days. At the end of the experiment, ıt was observed that HBO therapy increased osteoclastic activity and provided a significant increase in elimination of necrotic tissue, produced a significant erosion in living and necrotic regions in the subchondral bone, and no new bone formation was observed. The authors thought with these results that load on the femoral head may cause collapse, and recommended that HBO therapy should not be applied alone in the treatment of stage 2 AVN (13). In our study, elimination of the necrotic tissue and new bone formation were increased with HBO therapy, and better results were obtained in terms of remodeling and cartilage changes in the subchondral region compared to the control group. It was thought that different animals and re-repair processes, and different HBO therapy protocols applied might causes differences in osteoclastic activity, new bone formation and remodeling. It was thought that more than one session of Hbo a day as in the study by Jones et al. on rats, produced more bone breakdown due to highly increased osteoclastic activity. We think that femoral head should not be loaded together with HBO therapy in the treatment of AVN.

In a study by Peskin et al., induced AVN of the femur head in rats with the same of our model and examined in three groups (14). Load decreasing on the back legs method was used together with HBO therapy in Group 1, load decreasing on the back legs alone was performed in Group 2, and Group 3 was assigned as the control group. The animals received HBO therapy at 2.5 ATA for 90 minutes as 6 days in a week five days after the surgical procedure. The groups were divided into two with half was sacrificed at the 30th day and the other half at the 42th day. The necrosis in the femoral head observed at the 5th day, and symptoms seen later in humans were shown as the reason for treatment was started at the 5th day in rats. At the end of the study, no significant difference was found between the control group, and load decreasing group, while femoral heads showed less deformation, new bone formation was higher, and hematopoietic cells were better protected in the rats followed up for 30 days in the HBO therapy plus load decreasing group. No statistically significant difference was found between the group followed up

for 42 days with HBO therapy and the other groups. It was suggested that this result might be caused by toxic side effects due to higher stimulation of osteoclastic cells by oxygen radicals with prolonged HBO therapy (14). The two studies by Jones et al. and Barth et al. that we mentioned before this study were shown as examples. In our study, the rats were treated with HBO within 4 hours after surgical application for 30 days. Loading on the back legs was not restrained. HBO therapy was found to be efficient in terms of new bone formation, remodeling, and cartilage change at the end of 30 days compared to the control group.

Publications on the use of HBO therapy in clinical area are limited. In a study by Reis et al., 16 patients with Steinberg stage 1 AVN of the femoral head with lesion size being 4-12 mm in 12 patients were followed up with MRI examination. Patients with edema alone in the femoral head, and necrosis size less than 4 mm and greater than 12 mm were excluded from the study. Each patient in HBO therapy group received a total of 100 sessions with 2.0-2.4 ATA pure oxygen for 90 minutes. In MRI imaging performed before and after treatment, normal MRI imaging was obtained by 81% in HBO group and 17% in the control group. At the end of this study the authors reported that HBO therapy is a treatment method which can be used alone in Steinberg stage 1 AVN of the femur head (15). In another double blind randomized controlled study, Vezzani et al. treated 20 patients with Ficat stage 2 with 30 sessions of HBO therapy (2.5 ATA, 82 minutes/day) and hyperbaric air. Movements, stability and pain of the hip was tested during the treatment after the 10th, 20th and 30th sessions. Positive changes began in HBO therapy group in all parameters after the 20th session. Positive results could not be obtained in the group followed up with hyperbaric air.

None of the patients had complain of hip pain at the end of 6-year follow up. When MRI results in the beginning of the study were compared with the MRI results after 12 months, necrosis was shown to be completely disappeared in 7 of 9 patients (16). In a study investigating effects of Enoxaparin on a model that we have used, rats were followed up for 30 days as the control group and the group administered 1 mg/Kg IM Enoxaparin. The authors found that necrotic tissue was more regressed, remodeling was milder and cartilage change was lower in the Enoxaparin group compared to the control group. No significant difference was reported in new bone formation (4). The authors stated that lack of difference in new bone formation was an expected results caused by the known effects of Enoxaparin on bony tissue. In our study, new bone formation was higher, remodeling was milder and cartilage changes were lower in the group followed up for 30 days with Enoxaparin therapy compared to the control group. Lack of a statistically significant difference in the amount of necrotic tissue was attributed to small number of subjects in the groups.

In another study by Kang et al., individual and combined effects of Lovastatin and Enoxaparin were investigated in rats with experimental avascular necrosis induced by administration of methylprednisolone. Enoxaparin was administered in the animals as subcutaneous at a dose of 1 mg/Kg/day. When femur and humerus of the rabbits sacrificed at the end of the 2nd, 4th, 8th and 12th weeks were examined, the development of avascular necrosis was lower in the treatment group compared to the control group. The most significant effect was achieved with the combined therapy. Lower necrosis development was also observed in the groups administered Lovastatin and Enoxaparin therapies alone compared to the control group (17). In our study, Enoxaparin was not administered as prophylactic, and its effect was investigated in the subjects with necrosis induced. In our study also combined therapy with Enoxaparin and HBOT showed the best results. In a study with clinical use of Enoxaparin in the treatment of AVN, Glueck et al. compared patients with stage 1 and stage 2 AVN of the femur head which received Enoxaparin therapy (60 mg/Kg/day) with the control group of same feature which was not given any treatment. The patients were classified as type 1 including the patients who developed AVN due to thrombophilia or hypofibrinolysis, and type 2 including the patient who developed AVN due to use of steroids. At the end of 108 weeks, among 20 patients with AVN of the femur head, 19 were still at stage 1 or stage 2, while 12 of 15 patients with type 2 AVN progressed to Ficat stage 3 or stage 4. It was found in this study that the use of Enoxaparin in AVN developing due to thrombophilia or hypofibrinolysis prevented the development of necrosis (18).

In our study, results of the short term follow up of HBOT and Enoxaparin treatments were evaluated. HBOT and Enoxaparin therapies in AVN should be supported with another long term, controlled animal models. Although wwe think that addition of HBOT to AVN treatment protocol would be beneficial, further studies are needed with sessions of different number,

and duration and different pressure levels to determine its optimal effectiveness.

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