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A STUDY ON THE EFFECTS OF DIRECT FACTOR XA INHIBITORS AND DIRECT THROMBIN INHIBITORS ON HUMAN PRIMARY CHONDROCYTE CULTURES

Direkt Faktör Xa İnhibitörleri ve Direkt Trombin İnhibitörlerinin İnsan Primer Kondrosit Kültürlerine Etkileri Üzerine Bir Çalışma

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Abstract

Aim: This study investigates the effects of two direct factor Xa inhibitors, apixaban and rivaroxaban, and a direct thrombin inhibitor, dabigatran, on human primary chondrocyte cultures.

Materials and Methods: Monolayer cultured chondrocytes were prepared. Cell cultures were treated with dabigatran, apixaban, and rivaroxaban. Cultures without drug treatments served as the control group. Using an inverted light microscope, the cell surface morphology was examined. Cell viability and the toxicity of drugs were evaluated using a commercial assay kit, and the results were confirmed using two nucleic acid binding dyes, acridine orange and propidium iodide. The expressions of cartilage oligomeric protein, matrix metalloproteinase-7, and matrix metalloproteinase-19 were assessed using the real-time polymerase chain reaction analysis. All the analyses were performed within 21 days. The data obtained were statistically evaluated.

Results: The administration of the three drugs changed the cell viability, proliferation, and expressions of cartilage oligomeric protein, matrix metalloproteinase-7, and matrix metalloproteinase-19. The results were statistically significant (P<0.05).

Conclusion: Results obtained from in vitro studies may not provide accurate and reliable insight for clinical practices. However, clinicians should know that drugs used for the prevention or treatment of diseases may suppress chondrocyte proliferation and damage the extracellular matrix formation.

Keywords: Apixaban, chondrocyte, dabigatran, extracellular matrix, rivaroxaban.

Öz

Amaç: Bu çalışmada; direk faktör Xa inhibitörü apiksaban ve rivaroxaban ile Direk/selektif olarak thrombin inhibitörü olan dabigatranın, primer insan kıkırdak hücre kültürleri üzerine etkisinin gözlenmesi amaçlandı.

Materyal ve Metot: Kondrosit kültürleri monolayer olarak çoğaltılldı. Bu kültürler üzerine apiksaban, dabigatran ve rivaroksaban farmasötik ajanları ilave edildi. İlaç uygulanmayan hücre kültürleri kontrol grubu olarak kullanıldı. Hücrelere ait yüzey morfolojisi invert ışık mikroskobisi ile değerlendirildi. Hücrelerin sağlıklı olup olmadığı ve proliferasyonlarının devam edip etmediği MTT analizi ile spektrofotometrik olarak belirlendi ayrıca AO/PI fluresan boyamalardan da yararlanılarak apoptotik hücre ölümü varlığı araştırıldı. Kıkırdak oligo matriks protein (COMP), matriks metalloproteinaz (MMP)-7 ve MMP-19 genlerine ait ifadeler ise quantitative real time polymerase chain reaction (qRT-PCR) ile test edildi. Tüm analizler 21 gün içinde gerçekleştirildi. Elde edilen veriler istatistiksel olarak değerlendirildi ve sonuçlar raporlandı.

Bulgular: Bu üç farmakolojik ajanın, hem hücre canlılığı ve proliferasyonunu hem de COMP, MMP-7 ve MMP-19 genlerine ait ifadeleri değiştirdiği ve bu sonuçların istatistiksel olarak anlamlı olduğu kaydedildi (P<0.05).

Sonuç: Her ne kadar in-vitro deneylerden elde edilen sonuçlar klinik uygulamaları tam olarak yansıtmıyor olsa bile, herhangi bir hastalığı önleyebilmek amacı ile uygulanan ilaçların, kondrosit proliferasyonunu baskılayabileceği ve/veya ekstraselüler matrix (ECM) yapısına zarar verebileceği gerçeği akıllarda tutulmalıdır.

Anahtar Kelimeler: Apixaban, Dabigatran, Ekstraselüler Matriks, Rivaroksaban.

INTRODUCTION

Thromboembolic events are a leading cause of mortality. Alongside measures taken to prevent risk factors, pharmacological treatment modalities are also used to stop thrombotic events. Anticoagulants are used to prevent a new thrombus from forming or the growth of an existing thrombus. The relevant anticoagulant agents include heparin, low molecular weight heparin, parenteral direct thrombin inhibitors, fondaparinux, danaparoid, vitamin K

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antagonists, and new oral anticoagulant drugs (NOADs).

Apixaban, dabigatran, and rivaroxaban are known as NOADs or fixed-dose oral anticoagulants, and their clinical use has recently become widespread[1-3]. Apixaban is a novel, potent, reversible, competitive, direct factor Xa inhibitor, and prevents the conversion of prothrombin to thrombin. In randomized clinical trials, apixaban has been found to be more efficient in preventing stroke in non-valvular atrial fibrillation than warfarin and has widely been used in clinical practice since it causes less bleeding than warfarin[1].

Dabigatran, a direct thrombin inhibitor, is a prodrug that is orally absorbed through p-glycoprotein from the gastrointestinal tract and completely converted into its active metabolite by esterase-mediated hydrolysis in the liver, thereby providing safe and effective anticoagulation. Dabigatran etexilate does not use the cytochrome p-450 to convert its active metabolite[2,4].

Rivaroxaban is an oral, direct factor Xa inhibitor with a rapid onset of action[3]. NOADs are commonly used in clinics to reduce the risk of clot formation in atrial fibrillation and to prevent pulmonary embolism after hip or knee replacement surgery[5,6]. Patients using NOADs may experience side effects and adverse events, such as itchy rashes, redness of the skin, breathing difficulty, yellowing of the skin, yellowing of the sclera, and hemorrhage.

Many studies have researched the effects of dabigatran on intervertebral disc tissue[4]. No studies, however, have examined the effects of these kinds of pharmaceuticals on chondrocytes and extracellular matrix (ECM) formation. The present study investigates the effects of NOADs such as apixaban, dabigatran, and rivaroxaban on human primary chondrocyte cultures.

MATERIALS and METHODS

The present research was approved by the permission of the Local Ethics Committee of Istanbul Medipol University School of Medicine (29.11.2017-10840098/604.01.01/ E.44192). Written consent forms were obtained from all patients whose tissues were used in the preparation of the primary cell cultures.

Analyses were performed by the same researchers and repeated at least three times to minimize experimental errors. The researchers were blinded to the dosages and drugs, namely, the components in the culture medium.

Tissues extracted from the following patients were excluded: patients with fungal diseases who had used ketoconazole, itraconazole, voriconazole, or posaconazole for therapy; patients with human immunodeficiency virus (HIV) who had used lopinavir, ritonavir, or saquinavir for treatment; patients with immune-related diseases who had used cyclosporine and tacrolimus; and patients who had used anti-clotting drugs such as warfarin, enoxaparin, dalteparin, clopidogrel, ticagrelor, prasugrel, or aspirin.

The tissues of patients with gonarthrosis were used for the preparation of primary chondrocyte cultures. The primary cultures were fed every two days with freshly prepared culture medium [Dulbecco's Modified Eagle's Medium (DMEM; Cat. #41965062, Gibco) supplemented with 1% penicillin-streptomycin (PS; Cat. #15140122, Gibco), 15% fetal bovine serum (FBS; Cat. #10082147, Gibco), and 1%

L-glutamine (Cat. #25030081, Gibco)]. The drugs were administered to the culture medium following the third passage.

Tissues of patients with a grade 4 on the Kellgren-Lawrence scale were used who were unresponsive to the medical and conservative treatment (Figure 1).

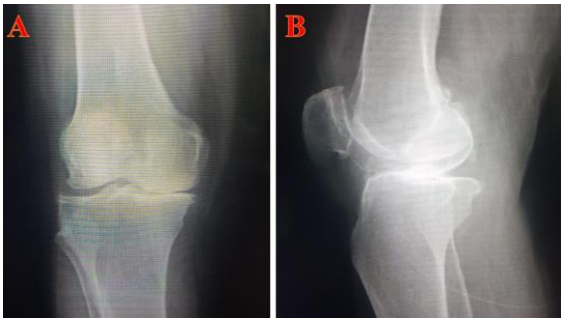

Figure 1. X-ray image of one of the operated patients.

The mean age of patients (n=6) whose osteochondral tissues were used was 66 (three males, three females, age range: 61-74). Osteochondral tissues were resected from the proximal and distal ends of the tibia and femur through a total knee arthroplasty. The resected tissues were placed in DMEM, then transferred to the laboratory to establish primary chondrocyte cultures[7,8]. Chondral tissues were separated from osteochondral tissues.

Osteochondral tissue samples were irrigated with 0.9% isotonic sodium chloride solution in a laminar flow cabinet to extract red blood cells. Tissues were dissected, washed in Hank's balanced salt solution (HBSS 1X; Cat. #14025, Gibco), and transferred to Falcon tubes. Collagenase type II enzymes (0.375 mg; Cat. #17101015, Gibco) that were dissolved in the complete medium were added to the solution and incubated in 5% CO2 at 37˚C overnight. Subsequently, the samples were centrifuged at 1,300 rpm for five min. The cell pellets were resuspended in the cell culture medium, transferred to T25 flasks, and incubated to obtain primary cell cultures[8].

Monolayer primary chondrocyte cultures were trypsinized and viable cells were determined through staining with trypan blue. Cell suspensions were plated at a density of $2x10^4$ cells/well in 96-well plates for (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) analysis, $2x10^4$ cells/well in 24-well plates for acridine orange (AO) / propidium iodide (PI) analysis, and $9x10^5$ cells/dish in 100-mm Petri dishes for RNA isolation. Following a 24 h incubation at 37˚C, drugs were added to these cell cultures (0 h).

NOADs were administered to the cultures established using tissue from hip replacement surgery for a period of 21 days and for a period of 15 days for knee replacement surgery. Analyses were performed at 0, 15, and 21 days following drug treatment.

Using an inverted light microscope (CKX41; Olympus Corporation, Tokyo, Japan), the cell surface morphology was examined under ×4, ×10, ×20, and ×40 magnifications. Cell viability and the toxicity of drugs administered were evaluated with a commercial MTT assay kit and the results were confirmed using the nucleic acid binding dyes AO and PI. A fluorescent microscope (DM2500; Leica Microsystems, Inc., Buffalo Grove, IL, USA) was used for the AO/PI analysis. Spectrophotometric absorbance was measured using a microplate reader (Mindray MR-96A; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

AO and PI may be used to accurately determine cell viability[8]. AO is an intercalating dye that permeates living and dead cells; it

stains all nucleated cells and generates green fluorescence[8]. PI can only enter dead cells with poor membrane integrity; it stains all dead nucleated cells to generate red fluorescence[8].

All the RNA was taken out from the cultured primary human chondrocytes using the PureLink RNA mini kit (Cat. #12183018A; Ambion, Thermo Fisher Scientific, Inc.) and 2-mercaptoethanol (Cat. #31350010; Thermo Fisher Scientific, Inc.). The quantity of RNA obtained from each sample was measured using a UV spectrophotometer (UV-VIS Spectrophotometer 2600; Shimadzu Corporation, Kyoto, Japan). To obtain cDNA, 50 ng of RNA was reverse transcribed using a high-capacity cDNA reverse transcription kit (Cat. #4368814; Thermo Fisher Scientific, Inc.) and a thermal cycler (ProFlex; Thermo Fisher Scientific, Inc.)[8].

The expression levels of cartilage oligomeric protein (COMP; Hs00164359_m1, Thermo Fisher Scientific, USA), matrix metalloproteinase-7 (MMP; TaqMan gene expression assays; Cat. #4331182), MMP-19 (TaqMan gene expression assays; Cat. #4331182), and internal control gene [housekeeping genes-actin beta (ACTB; Cat. #4453320)] were assessed using gene-specific TaqMan Gene Expression Assays. The genes were detected using the real-time polymerase chain reaction (RT-PCR) analysis.

For obtaining comparative results, a reference (calibrator) sample (0 h) was used and relative quantity (RQ) values were calculated using the $2^{\Delta\Delta Cq}$ method.

RESULTS

Cell viability decreased by 10.57%, 12.37%, and 16.15% in the samples treated with dabigatran, apixaban, and rivaroxaban, respectively.

Cell counts significantly decreased in the samples treated with apixaban and rivaroxaban on day 21, while no viable cell was observed in the dabigatran-treated samples (Table 1).

Table 1. MTT cell viability, toxicity, and proliferation assay.

	Apixaban (mean±SD)	Dabigatran (mean±SD)	Rivaroxaban (mean±SD)	F-Value	P-Value*
Control, 0 h	0.5±0.01	0.2452±0.00	0.4155±0.00	6.76	0.003
Application, 0 h	0.5±0.01	0.2452±0.06	0.4155±0.00		
Control, day 15	3.5583±0.01	2.4714±0.01	0.4155±0.02	5.62	0.008
Application, day 15	0.4402±0.01	0.2613±0.01	0.6261±0.01		
Control, day 21	7.4561±0.02	5.01±0.02	7.6836±0.02	7.55	0.000
Application, day 21	0.459±0.01	0.000±0.00	0.5133±0.01		

*ANOVA, analysis of variance, SD; standard deviation.

The results of the MTT assay were confirmed using AO and PI (Figure 2).

No COMP expression was observed in the dabigatran-treated samples on day 15. COMP expression decreased 0.31-fold in the apixaban-treated samples on day 21, while it increased 11.05-fold and 2.86-fold in the samples treated with dabigatran, and rivaroxaban, respectively. MMP-7 expression increased 42.44-fold in the apixaban-treated samples when compared to the control group samples. The RQ value of MMP-7 increased 5.48-fold in the rivaroxaban-treated samples. MMP-19 expression decreased 0.5-fold in the apixaban-treated samples. The RQ value of MMP-19 decreased 0.34-fold in the rivaroxaban-treated samples when compared to the control group samples. MMP-7 and MMP-19 expressions were eliminated on days 15 and 21 with the dabigatran treatment (Figure 3).

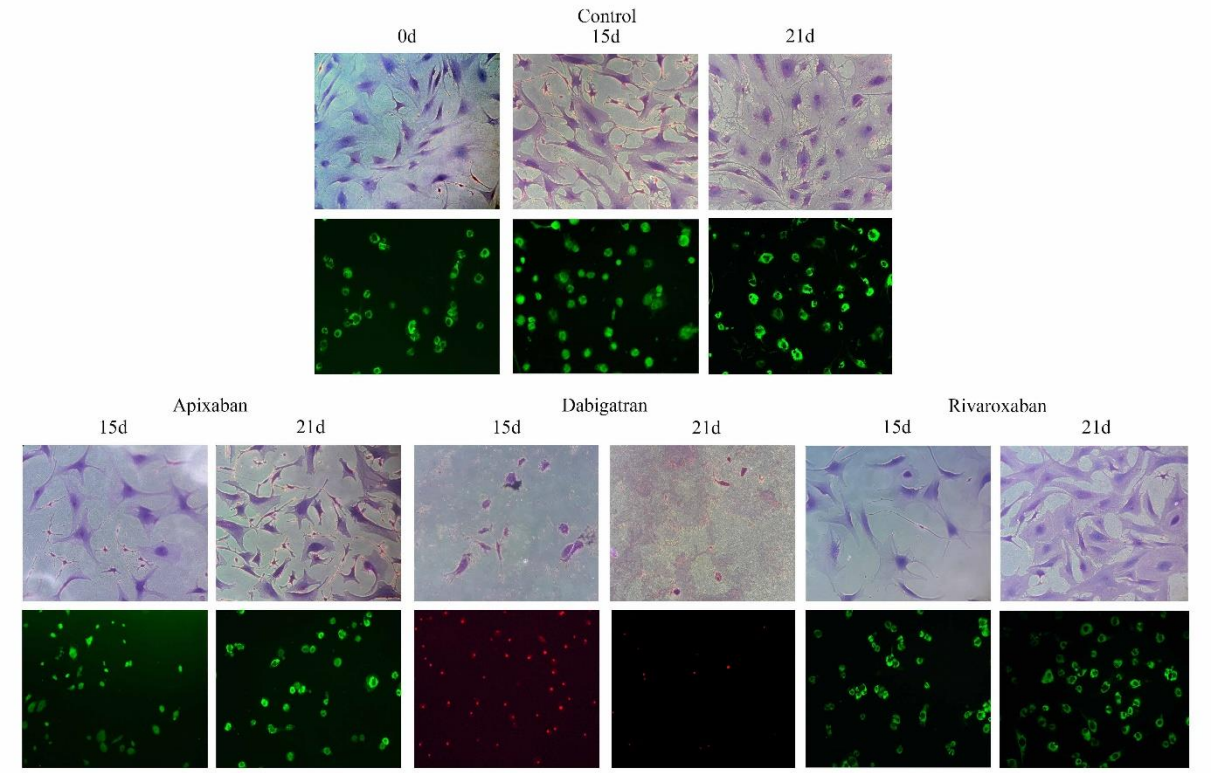

Figure 2. Images of AO/PI staining obtained using a fluorescent microscope.

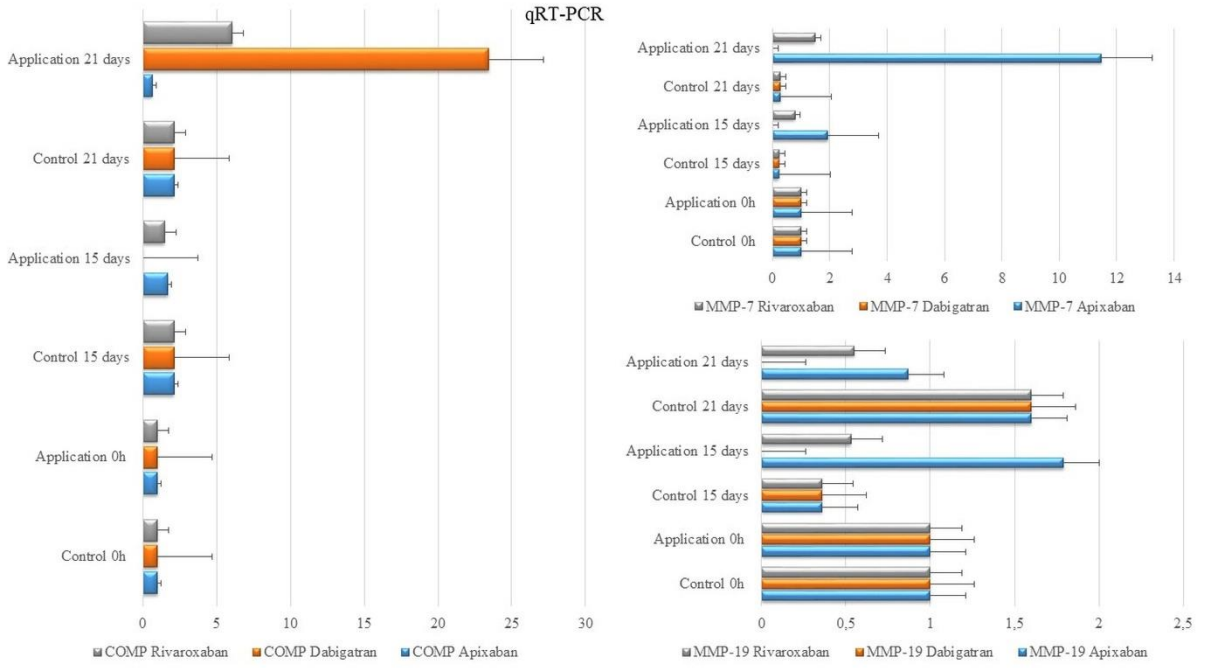

Figure 3. The expression levels of COMP, MMP-7 and MMP-19 in control group samples and samples treated with apixaban, dabigatran, and rivaroxaban.

DISCUSSION

Cartilage tissue is avascular, aneural, and devoid of lymph tissues[8-11]. Thus, cartilage cells are fed by a perichondrium layer in the vascular structure or by synovial fluid that washes the articular surfaces without perichondrium[9-12]. Since the outer layer of the synovial tissue is thick, a considerable number of drugs and nutrients are diffused from synovial tissue into the synovial fluid[9-13]. Then, they pass through pores and reach chondrocytes where a second diffusion occurs[9-14]. Many drugs, whether given orally or

parenterally, are known to accumulate in the synovial fluid compartments[9-16].

Osteoarthritis is a multifactorial disease characterized by the degeneration of articular cartilage. A number of metabolic, genetic, and related factors may cause osteoarthritis; however, its etiology is still unknown. Drugs used for its treatment may also aggravate the degeneration of cartilage.

COMP is a well-known marker of cartilage breakdown. An elevated level of COMP predicts the progression of osteoarthritis. Since COMP is synthesized not only by cartilage, but also by synovial cells like tendon fibroblasts and osteoblasts, it may increase because of cartilage destruction or synovial inflammation. In knee osteoarthritis, the level of COMP is compatible with synovitis grade, but it is not compatible with the grade. The lack of COMP specificity may restrict its usage in evaluating changes in joint damage in osteoarthritis and rheumatoid arthritis[17,18]. In the present study, the dabigatran and rivaroxaban treatments increased the level of COMP.

Metalloproteinase enzymes may degrade ECM components by affecting both aggregate and type II collagen. Some studies have suggested that MMP-7 may play a significant role in the pathogenesis of osteoarthritis and it has a positive correlation with osteoarthritis[19,20]. A study has reported that an increase in the MMP-19 level is associated with the pathogenesis of osteoarthritis and causes damage to cartilage tissue[21].

In the present study, no COMP expression was observed in the dabigatran-treated samples on day 15, while COMP expression increased 11.05-fold on day 21 when compared to the control group samples. A decrease in COMP expression was correlated with the morphological changes. COMP expression decreased 0.31-fold in the apixaban-treated samples on day 21, while it increased 2.86-fold in the rivaroxaban-treated samples. MMP-7 expression increased 42.44-fold in the apixaban-treated samples when compared to the control group samples. The RQ value of MMP-7 increased 5.48-fold in the rivaroxaban-treated samples. MMP-19 expression decreased 0.5-fold in the apixaban-treated samples. MMP-7 and MMP-19 expressions were eliminated on days 15 and 21 after dabigatran treatment. The RQ value of MMP-19 decreased 0.34-fold in the rivaroxaban-treated samples when compared to the control group samples. In the present study, changes in COMP, MMP-7, and MMP-19 expressions were observed after drug treatment. The results obtained revealed that the administered drugs might adversely affect tissue healing. Decreases in proliferation, increases in apoptosis, and changes in gene expressions should be considered to provide more accurate and reliable data.

The present study has a number of limitations. The cell cultures were obtained from a small number of patients, who were all of the same race. However, three cell cultures were established for each patient and all the experiments were repeated three times. Gene expressions may alter depending on individual differences. Therefore, analyses should be carried out using tissue from a larger number of participants from different races. The changes in gene expressions that were observed using the RT-PCR analysis may provide comprehensive and reliable data. The fact that dabigatran etexilate is an oral prodrug was a limitation of the study because it is converted into dabigatran, a reversible, direct,

competitive thrombin inhibitor, by a serum esterase in the body and this study was not performed *in vivo*, but rather *in vitro*.

Cell viability and proliferation decreased in the human primary chondrocyte cultures separately treated with three drugs on day 21. The drug administration changed the expressions of COMP, MMP-7, and MMP-19. The dabigatran and rivaroxaban treatments increased the level of COMP, which causes osteoarthritis and rheumatoid arthritis. An overexpression of MMP-7 and MMP-19 may lead to catabolic reactions. In the present study, MMP-7 and MMP-19 were overexpressed in the apixaban and rivaroxaban-treated samples.

CONCLUSION

The use of NOADs has recently been proposed as an alternative to vitamin K antagonists, which has long been a gold standard therapy to prevent stroke after knee and hip replacement surgery or in patients with atrial fibrillation. The increasing use of NOADs has resulted in significant problems and clinical conditions. The results obtained from this study revealed another medical problem with these drugs. Although the study was performed *in vitro*, the results showed that even a single application of each drug caused changes in proliferation, cell viability, and gene expression, thereby affecting the ECM formation. Clinicians should be cautioned about the adverse and toxic effects of NOADs and they should pay attention to their application dosage and time.

References

1. Ozer N. Clinical studies conducted with new oral anticoagulants in atrial fibrillation: Which oral anticoagulant can be considered for which case in light of the clinical studies? Turk Kardiyol Dern Ars. 2016;44 Suppl 2:33-40.
2. Deveci OS, Demir M, Aksoy M. Use of dabigatran in patients with non-valvular atrial fibrillation: answers to frequently asked questions. Arch Turk Soc Cardiol. 2013;41 Suppl 4:25-33.
3. Bas DF, Topcuoglu MA, Arsava EM. Atrial fibrillation and stroke in the perspective of new oral anticoagulants. Turkish Journal of Cerebrovascular Diseases. 2013;19(2):35-45.
4. Kaplan N, Karaarslan N, Yilmaz I, Sirin DY, Akgun FS, Caliskan T, et al. Are intervertebral disc tissue cells damaged when attempting to prevent thrombus formation using dabigatran, a new oral anticoagulant? Turk Neurosurg. 2019;29(4):470-477.
5. Coleman CI, Peacock WF, Bunz TJ, Alberts MJ. Effectiveness and safety of apixaban, dabigatran, and rivaroxaban versus warfarin in patients with nonvalvular atrial fibrillation and previous stroke or transient ischemic attack. Stroke. 2017;48(8):2142-2149.
6. Torrejon Torres R, Saunders R, Ho KM. A comparative cost-effectiveness analysis of mechanical and pharmacological VTE prophylaxis after lower limb arthroplasty in Australia. J Orthop Surg Res. 2019;14(1):93.
7. Isyar M, Yilmaz I, Yasar Sirin D, Yalcin S, Guler O, Mahirogullari M. A practical way to prepare primer human chondrocyte culture. J Orthop. 2016;13(3):162-7.
8. Gumustas SA, Yilmaz I, Isyar M, Sirin DY, Batmaz AG, Ugras AA, et al. Assessing the negative impact of phenyl alkanoic acid derivative, a frequently prescribed drug for the suppression of pain and inflammation, on the differentiation and proliferation of chondrocytes. J Orthop Surg Res. 2016;11(1):70.
9. Guzelant AY, Isyar M, Yilmaz I, Sirin DY, Cakmak S, Mahirogullari M. Are chondrocytes damaged when rheumatologic inflammation is suppressed? Drug Chem Toxicol. 2017;40(1):13-23.
10. Gumustas F, Yilmaz I, Sirin DY, Gumustas SA, Batmaz AG, Isyar M, et al. Chondrocyte proliferation, viability and differentiation is declined following administration of methylphenidate utilized for the treatment of attention-deficit/hyperactivity disorder. Hum Exp Toxicol. 2017;36(9):981-992.
11. Dogan M, Isyar M, Yilmaz I, Bilir B, Sirin DY, Cakmak S, et al. Are the leading drugs against Staphylococcus aureus really toxic to cartilage? J Infect Public Health. 2016;9(3):251-258.
12. Karaarslan N, Yilmaz I, Sirin DY, Ozbek H, Kaya YE, Akyuva Y, et al. Does transcription factor, induced by daptomycin and vancomycin, affect HIF-1α, Chondroadherin, and COL2A1? Ann Med Res. 2018;25(4):756-62.
13. Sirin DY, Kaplan N, Yilmaz I, Karaarslan N, Ozbek H, Akyuva Y, et al. The association between different molecular weights of hyaluronic acid and CHAD, HIF-1α, COL2A1 expression in chondrocyte cultures. Exp Ther Med. 2018;15(5):4205-4212.
14. Karaarslan N, Batmaz AG, Yilmaz I, Ozbek H, Caliskan T, Yasar Sirin D, et al. Effect of naproxen on proliferation and differentiation of primary cell cultures isolated from human cartilage tissue. Exp Ther Med. 2018;16(3):1647-1654.
15. Sirin DY, Karaarslan N. Evaluation of the effects of pregabalin on chondrocyte proliferation and CHAD,

HIF-1α, and COL2A1 gene expression. Arch Med Sci. 2018;14(6):1340-1347.
16. Kaplan N, Yilmaz I, Karaarslan N, Kaya YE, Sirin DY, Ozbek H. Does nimodipine, a selective calcium channel blocker, impair chondrocyte proliferation or damage extracellular matrix structures? Curr Pharm Biotechnol. 2019;20(6):517-524.
17. Punzi L, Oliviero F, Plebani M. New biochemical insights into the pathogenesis of osteoarthritis and the role of laboratory investigations in clinical assessment. Crit Rev Clin Lab Sci. 2005;42(4):279-309.
18. Boeth H, Raffalt PC, MacMahon A, Poole AR, Eckstein F, Wirth W, et al. Association between changes in molecular biomarkers of cartilage matrix turnover and changes in knee articular cartilage: a longitudinal pilot study. J Exp Orthop. 2019;6(1):19.
19. Tao Y, Qiu X, Xu C, Sun B, Shi C. Expression and correlation of matrix metalloproteinase-7 and interleukin-15 in human osteoarthritis. Int J Clin Exp Pathol. 2015;8(8):9112-9118.
20. Chang ZK, Meng FG, Zhang ZQ, Mao GP, Huang ZY, Liao WM, et al. MicroRNA-193b-3p regulates matrix metalloproteinase 19 expression in interleukin-1β-induced human chondrocytes. J Cell Biochem. 2018;119(6):4775-4782.
21. Papathanasopoulos A, Kouroupis D, Henshaw K, McGonagle D, Jones EA, Giannoudis PV. Effects of antithrombotic drugs fondaparinux and tinzaparin on in vitro proliferation and osteogenic and chondrogenic differentiation of bone-derived mesenchymal stem cells. J Orthop Res. 2011;29(9):1327-35.