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Research Article
Effects of Tocilizumab on Intervertebral Disc Degeneration, Cell Senescence and Inflammation via BMP-2, Hif-1α , IL-1β and SOX9

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Abstract

Background and Objective: Immunosuppressive tocilizumab (TCZ), which is frequently used in the treatment of rheumatoid arthritis, can have many side effects as well as an uncontrolled inflammatory response. This study aimed to evaluate the effect of tocilizumab (TCZ) administered to intervertebral disc (IVD) tissues *in vitro* on the proinflammatory cytokines and proteins of degeneration, senescence and inflammation-related signaling pathways at the pharmaco-molecular level. **Materials and Methods:** Primary cell cultures were prepared using human IVD tissues obtained during lumbar microdiscectomy. Untreated groups served as the control and TCZ-treated groups as the study sample. Analyses were performed using a commercial kit, supravital and fluorescent dyes. Changes in bone morphogenetic protein (BMP)-2, hypoxia-inducible factor (Hif)1-alpha (Hif1-α), interleukin (IL)-1 beta (IL-1β) and sex-determining region Y (SRY)-box 9 (SOX9) protein expressions were evaluated using western blotting. An alpha value of less than 0.05 was considered significant. **Results:** Proliferation decreased in the samples treated with TCZ (10 μg mL^{-1}) on day 15 ($p<0.05$). Protein expressions of BMP-2, Hif-1α, IL-1β and SOX9, which play a vital role in anabolic and catabolic pathways, changed in samples treated with TCZ (10 μg mL^{-1}). **Conclusion:** This change was statistically significant ($p<0.05$). Therefore, results concluded that the inflammation, extracellular matrix degradation and nucleus pulposus degeneration after disc herniation are controlled by BMP-2, Hif-1α, IL-1β and SOX9.

Key words: BMP-2, Hif-1α, IL-1β, SOX9, tocilizumab, disc herniation, proinflammatory cytokines, oral herpes simplex, morphogenetic protein-2

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tocilizumab (TCZ), also known as atlizumab, is an immunosuppressive drug used for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis, a serious type of arthritis seen in children[1]. This drug is a monoclonal antibody against the interleukin (IL)-6 receptor[2]. However, studies performed during the COVID-19 pandemic[3,4] established that this monoclonal antibody could be an important option, especially in the treatment of severe COVID-19 pneumonia. Some studies[5-7] have suggested that TCZ can suppress an uncontrolled inflammatory response.

Any drug given at regular doses to prevent, diagnose or treat a disease or to improve, restore or change a physiological function could cause harmful and unintended effects, called adverse effects[8]. The intervertebral disc (IVD) is an avascular tissue under hypoxic conditions after adulthood[9]. Previous studies have shown that proinflammatory cytokines IL-1β and bone morphogenetic protein (BMP)-2 play important roles in the healing process after IVD degeneration (IVDD)[10]. The IVD tissue is fed with synovial fluid. Drugs accumulate in the synovial fluid whether they are taken orally or parenterally. Many drugs taken into the body accumulate in the synovial fluid compartment[11].

The TCZ can produce many side effects such as fever, nausea, diarrhoea, constipation, headache, dizziness, cough, pneumonia, herpes zoster, oral herpes simplex, skin infections with fever and chills, Stevens-Johnson syndrome, conjunctivitis, neutropenia, leukopenia, diverticulitis, hypersensitivity and fatal anaphylaxis[12]. However, no studies have investigated the effects of TCZ on annulus fibrosus (AF)/nucleus pulposus (NP) cells, extracellular matrix (ECM) structure or IVD.

According to a review that evaluated degeneration and inflammation, in senescent and dedifferentiated chondrocytes. It was found that bone morphogenetic protein-2 (BMP-2) and sex-determining region Y (SRY)-box 9 (SOX9) are downregulated, while the levels of interleukin (IL)-1 beta (IL-1β), is upregulated when the normal molecular function of the chondrocytes are inhibited[13].

The SOX9 functions in the regulation of the cytoskeleton and ECM by acting on many signalling pathways related to metabolic pathways, ion transport and cell cycle in NP and AF cells[14]. The SOX9, which plays an important role in chondrocyte differentiation, protects the boundaries of the notochord and IVD compartments in the developing axial skeleton. Decreased SOX9 expression is associated with campomelic dysplasia and results in severe scoliosis and progressive IVDD[14].

The BMP-2 has positive effects on the telomerase activity, replication capacity and senescence of NP cells[15]. Both *in vivo* and *in vitro* experiments proved the anti-apoptotic effect of hypoxia-inducible factor (Hif)1-alpha (Hif-1α) on NP-derived stem cells under excessive mechanical loading, suggesting that restoring hypoxia and manipulating autophagy are crucial to maintaining intrinsic repair and retarding IVDD[16]. The Hif-1α is vital for the survival of NP cells and chondrogenesis is regulated by numerous mechanisms, including growth factors such as Hif-1α, a helix-loop-helix transcription factor that is expressed in hypoxic conditions[17].

This study aimed to assess the cellular effects of TCZ on human primary IVD tissue treated with TCZ. In addition, the study asked whether TCZ could affect senescence mechanisms associated with degeneration and inflammation in IVD tissue through protein expression of the BMP-2, IL-1β, SOX9 and Hif-1α signaling pathways.

MATERIALS AND METHODS

Study area: This study was carried out in cultures prepared from the tissues of the operated cases between 23-02-2022 to 23-05-2022 at Halic University of the School of Medicine. Pharmaco molecular analyzes were performed in the Molecular Biology and Genetics Laboratory, Namık Kemal University, Turkey.

To minimize bias, all analyses were carried out by the same researcher. All the experiments were performed three times.

Case selection criteria and preparation of primary cell cultures: The tissues of patients with neutropenia, leukopenia, or thrombocytopenia; patients with active tuberculosis or active hepatitis B or C and pregnant women were not used in the preparation of the primary cell cultures. The tissues of patients with an allergy to oseltamivir were also excluded from the study. The IVD tissues were obtained from patients (Pfirrmann grading scale stage III-IV; 4 females, 4 males; mean age: 42.18±7.62 years) diagnosed with lumbar disc herniation but who did not respond to conservative medical treatment[18].

Primary cell cultures were prepared using extruded or migrated disc fragments obtained during lumbar microdiscectomy and lumbar micro-sequestrectomy. The tissues were transferred to the laboratory at 4°C in a cell culture medium containing 1% penicillin-streptomycin. The tissues that were irrigated and washed in a laminar flow cabinet (Air Flow-NUVE/NF-800 R, Ankara, Turkey) were then transferred to Petri dishes separately and mechanically

disintegrated using a rongeur. They were enzymatically degraded using a collagenase type II enzyme/collagenase type I enzyme dissolved in Hank's Balanced Salt Solution (HBSS). After overnight incubation at 37°C with 5% CO_2, the samples were centrifuged at 4°C and 1300 rpm twice consecutively for 10 min. The supernatant on the tubes was discarded. Cell pellets were resuspended with a fresh cell culture medium. The samples were then transferred to flasks. Confluent primary cell cultures were passaged three times. In the third passage, the cells were trypsinized with 0.25% trypsin-ethylenediaminetetraacetic and counted using the Thoma slide. The counted cells were plated at 1.6×10^4 cells per well in 96-well plates for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability and proliferation and toxicity assays, acridine orange (AO) and propidium iodide (PI) (AO/PI) staining, Janus Green B staining and western blotting evaluations, respectively. All the samples were then incubated in an incubator set to 37.4°C at 5% CO_2 and the experiments were performed.

Drug preparation and application to human primary chondrocyte cultures: The vial (Actemra® 80 mg/4 mL IV; Chugai Pharma Manufacturing Co. Ltd., Utsunomiya-City, Japan) containing 80 mg of TCZ (20 mg mL^{-1}) in 4 mL of solution was mixed with 96 mL of sterile 0.9% isotonic sodium chloride infusion solution and a 100 mL final stock solution was obtained. Dilution was performed by adding Dulbecco's Modified Eagle's Medium at a ratio of 1:1. The resulting TCZ concentration was 10 μg mL^{-1}. Untreated samples served as a control group and were named group 1. Samples treated with TCZ served as a study group and were named group 2.

Pharmaco molecular analyses: The half-life of TCZ is concentration-dependent and its effect can persist for several weeks after the end of treatment[19]. All molecular analyses were performed simultaneously on days 1, 7 and 15 due to the long half-life of TCZ and the persistence of its effect after drug discontinuation.

Evaluations via inverted light microscopy: The AF/NP cell surface morphologies and ECM structures were examined under 4×, 10×, 20× and 40× magnifications using an inverted light microscope. Microphotographs of cell organization were obtained and analyzed during the confocal/contrast phase using Olympus Cell Soft Imaging System software.

Cell viability, toxicity and proliferation analyses using MTT and enzyme-linked immunosorbent assay: The viability tests were carried out using an MTT kit, which inhibits formazan crystal formation in dead cells[20]. The Vybrant MTT cell proliferation assay was from Cell Biolabs Inc. (USA). For viability and cell toxicity measurements, a Mindray MR 96 ELISA device, from PRC, was used. Absorbance was then measured at a wavelength of 540 nm.

AO, PI and Janus Green-B staining: The AO produces green fluorescence by staining all nucleated cells[21], whether alive or dead, whereas PI penetrates only dead cells with poor membrane integrity and stains nucleated cells to produce red fluorescence. The mitochondria and DNA damage were determined using Janus Green-B dye, which changes colour according to the amount of oxygen. The indicator oxidizes to a blue colour when oxygen is present. In the absence of oxygen, the indicator decreases and the colour changes to pink[22].

Analysis of BMP-2, Hif-1α, IL-1β and SOX9 protein expressions by western blotting: For the total protein isolation, the human primary IVD cell culture samples were placed in encoded eppendorf tubes containing a Triton X-100 (Sigma-Aldrich, Cat no. 10789704001, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) protease inhibitor cocktail (Roche, Cat no. 04693159001, Taufkirchen, Germany). The protein lysates were processed using a western blot test to reveal the expressions of BMP-2 (Cat no. MA523764, Thermo Fisher, Waltham, MA, USA), Hif-1α (Cat no. MA1516, Thermo Fisher, Waltham, MA, USA), IL-1β (Cat no. M421B, Thermo Fisher, Waltham, MA, USA) and SOX9 (Thermo Fisher Scientific, Cat no. 14-9765-82, Waltham, MA, USA).

The total protein amount was determined using the Bradford protein assay kit. Proteins of the samples, each of which contained 100 mg of protein were separated using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Using iBlot, the samples were transferred to a Polyvinylidene Difluoride (PVDF) membrane (Thermo Fisher Scientific, Cat no. IB401001, Waltham, MA, USA) to perform immunoblotting[20,21].

Monoclonal β-actin antibody (Cat no. MA511869, Thermo Fisher, Waltham, MA, USA) was used as the housekeeping gene. Immunoblotting was performed using a Western Breeze chemiluminescence kit (Thermo Fisher Scientific, Cat no. WB7104, Waltham, MA, USA) according to the manufacturer's instructions. The protein bands transferred to an X-ray film (Thermo Fisher Scientific, Cat no. 34090, Waltham, MA, USA) were analyzed using ImageJ software and the specific amount of protein in each sample was determined[22-24].

Statistical analysis: Minitab software (version 22) was used for the statistical evaluation. Data were evaluated at a 95%

confidence interval (CI). Analysis of Variance (ANOVA) was used to determine the differences between groups. Tukey's honest significant difference (HSD) test was used to assess differences between multiple groups. The results were presented as Mean±Standard Deviation (mean±SD). An alpha value less than 0.05 was assumed to be significant.

RESULTS

The AF and NP cell surface morphologies and ECM structures were examined using an inverted light microscope. It was observed that the cells maintained their specific morphology during the application period and they were also able to form ECM. Cultures were also visualized by staining with Janus Green B for more detailed examinations. The primary cultures formed consist of shuttle-like AF cells and relatively larger NP cells with more abundant cytoplasm (Fig. 1(a-l)).

All cultures were also stained with AO and PI and it was evaluated whether the drug had a cytotoxic effect at the applied dose. As a result, evaluation of the primary IVD tissue cultures indicated that administration of the drug did not cause changes in cell morphology. Further, no cytotoxicity was observed following drug administration.

Although TCZ did not show cytotoxic effects in AF and NP cultures, it suppressed cell proliferation. Proliferation decreased by 18.15% in the TCZ-treated samples compared with the control samples. In turn, proliferation decreased by 37.49 and 56.18% on days 7 and 15, respectively, in the TCZ-treated samples compared with the control samples. The results obtained were statistically significant ($p<0.05$). The results of Tukey's HSD and ANOVA in treated and

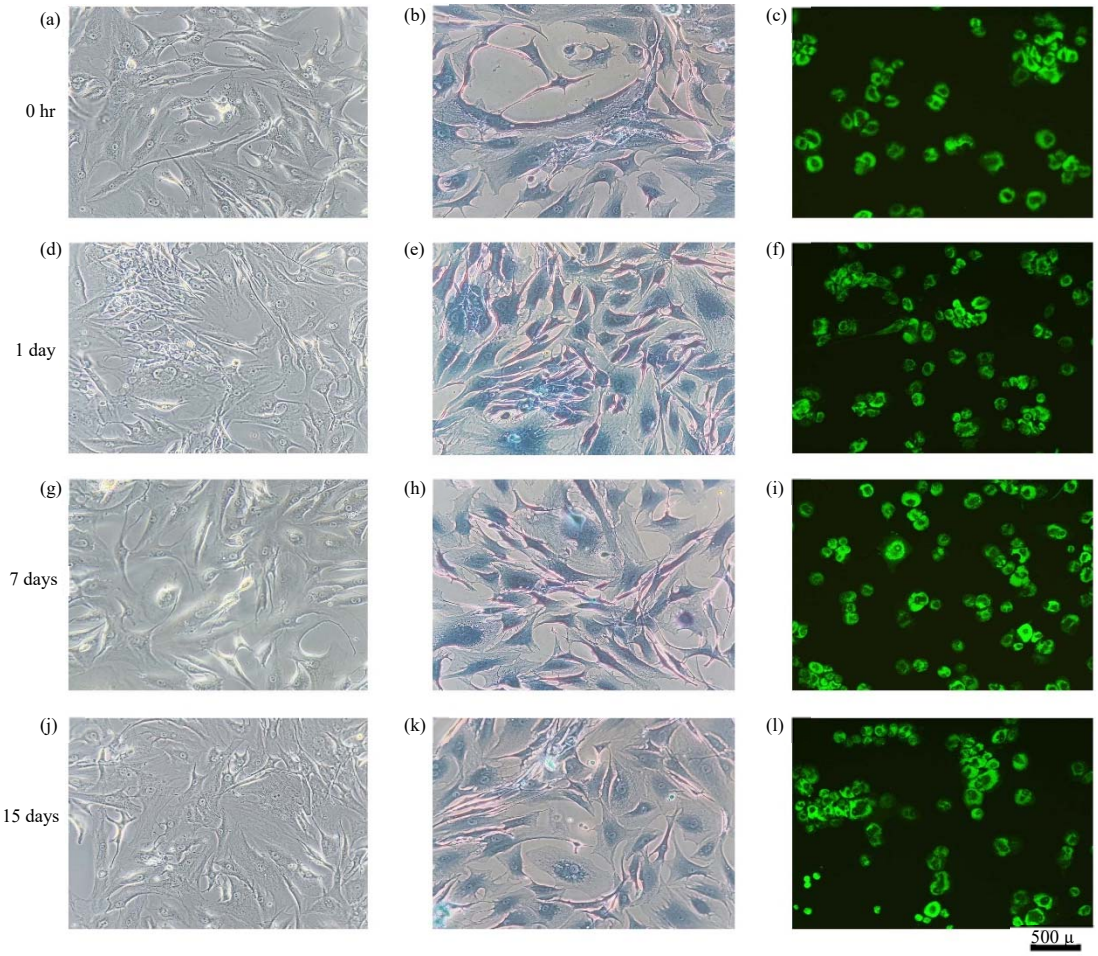

Fig. 1(a-l): Morphological examination of primary cell culture samples with and without TCZ, the first lane (a-c) Control group samples, (d-f) Micrographs of tocilizumab-applied cultures for 1 day, (g- l) 7 days and (j-l) 15 days
First column, inverted microscopy: Second column, Janus Green-B-stained cultures (20× magnification): Third column, acridine orange/propidium iodide-stained (10× magnification) AF and NP cells cultures

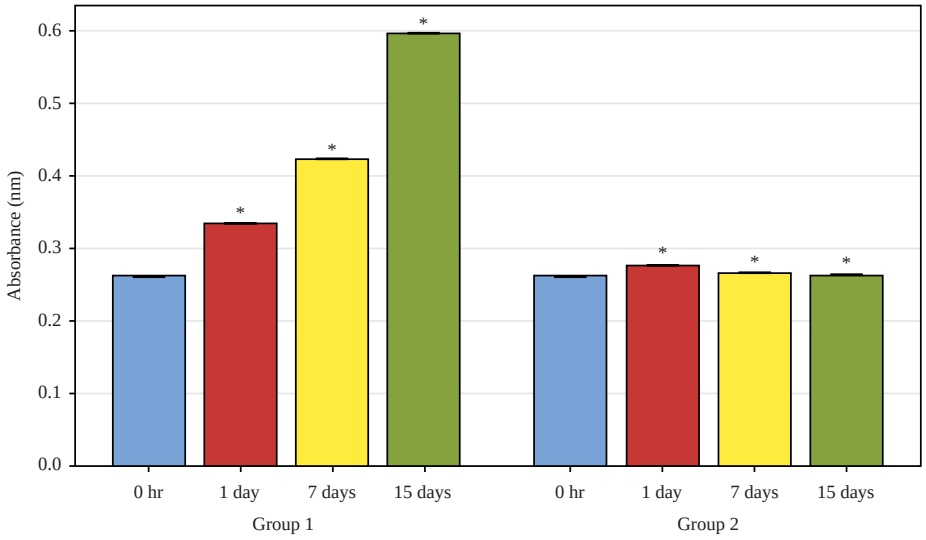

Fig. 2: Effects of tocilizumab treatment on the proliferation of intervertebral disc tissue cells

Data were analyzed using a one-way analysis of variance followed by Tukey's *post hoc* test, *statistically significant and p<0.05

Table 1: Assessment of the cell viability, toxicity and proliferation of intervertebral disc tissue cells following tocilizumab treatment

Source	Adj SS	Adj MS	F-value	p-value*
Groups	0.231296	0.231296	190106.58	0.000
Time (days)	0.185119	0.061706	50717.63	0.000
Groups*Time (days)	0.193408	0.064469	52988.58	0.000

*p<0.05 vs control group and p<0.05 vs TCZ-treated group, data were analyzed using a one way analysis of variance, Adj SS, an adjusted sum of squares and Adj MS, adjusted mean square

Table 2: Comparison of the absorbance values of viability in intervertebral disc cell cultures treated with tocilizumab with the control group

Groups vs Time	Time (days)	Mean±St. Dev	Grouping*
Group1	15	0.598±0.001	A
Group 1	7	0.424±0.002	B
Group 1	1	0.336±0.001	C
Group 2	1	0.275±0.001	D
Group 2	7	0.265±0.001	E
Group 2	15	0.262±0.001	F
Group 1	0	0.261±0.001	F
Group 2	0	0.261±0.001	F

*A: Tukey's honest significant difference test, the highest rate of cell viability and proliferation and F: The lowest rate of cell viability and proliferation

Table 3: Protein expression values in terms of fold change (r)

Application duration	β-Actin	BMP-2	Hif-1α	Sox9	IL-1β
0 hr	1	1	1	1	1
1 day	1	1.66	1.51	2.77	3.77
7 day	1	1.93	2.71	3.40	3.95
15 day	1	1.54	1.28	1.99	4.04

untreated primary cell cultures were presented in Table 1,2 and Fig. 2.

The results were expressed as fold change (increase or decrease). The values were taken 100% or r = 1-fold at 0 hr. The BMP-2, Hif-1α, SOX9 and IL-1β increased by 1.66, 1.51, 2.77 and 3.77 times, respectively, in the TCZ-treated IVD samples on day 1. The BMP-2, Hif-1α, SOX9 and IL-1β increased by 1.93, 2.71, 3.40 and 3.95 times, respectively, on day 7. Likewise, BMP-2, HIF-1 α, SOX9 and IL-1β increased by 1.54, 1.28, 1.99 and 4.04 times, respectively, on day 15 (Table 3, Fig. 3).

In the continuation of the study, some of the molecular pathways underlying the suppression of cell proliferation were evaluated. Total protein was obtained from all experimental groups and the expression levels of BMP-2, Hif-1α, SOX9 and IL-1β proteins were compared by the

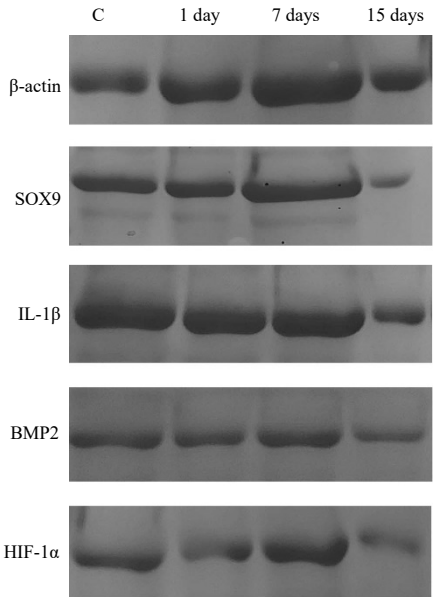

Fig. 3: Western blot analysis of SOX9, IL-1β, BMP-2 and HIF-1α

β-actin was used as an internal control gene, the figure demonstrates SOX9, IL-1β, BMP-2 and HIF-1α expressions determined with western blot, protein lysates used in western blot were obtained from AF and NP cells cultures at 0 hr (control group, no application, first column), the second column at 1 day, the third column at 7 days and the fourth column at 15 days of tocilizumab application

western blot method. The amount of protein in all samples was normalized with the are given in the supplementary data (Fig. S4a-e).

DISCUSSION

Many studies have reported the side effects of TCZ. However, no studies have yet investigated the effects of TCZ on AF and NF cells that form IVD. Experimental research that examines drug cytotoxicity generally uses commercial cell lines or animal tissues[24,25].

Commercial cell lines contain only one cell type and lack complex coordination mechanisms in the cells' microenvironment. They do not have the same genotypic and/or phenotypic characteristics as in the human body; therefore, the results of studies using cell lines could be misleading[26]. The sensitivity of animal tissue is known to differ from that of human tissue[26]. Therefore, the results obtained from assays using animal tissues could diverge from those using human tissues, which might result in misleading outcomes[23,26].

In the present study, primary cell cultures prepared from human degenerated IVD were used, which could enhance its value.

In the literature, the importance of the SOX9 gene in ECM anabolism and NP cell senescence is emphasized[27].

In mice, SOX9 deletion causes IVDD and changes the phenotype of disc cells and the ECM structure. The SOX9 regulates distinct transcriptomic landscapes in IVD tissue compartments[14]. The SOX9 controls cell survival and differentiation in the inner AF during IVD development[14].

It has been reported that the inflammatory response in the IVD tissue can be limited by the SOX9 elevation, in this way slowing the development of apoptosis and IVDD in degenerative NP cells[28].

The SOX9 gene was targeted in NP cells to regulate the expression of proinflammatory factors such as IL-1β, TNF-α, IL-6 and prostaglandin-2[29].

In this study, SOX9 protein expression increased by 99% in primary cell cultures treated with TCZ compared with the control samples. The AF/NF cell proliferation decreased by 56.18% in the TCZ-treated samples compared with the control samples on day 15. Although the cell proliferation decreased at the end of the 15th day, the protein expression of SOX9 increased, in the TCZ-treated cell groups.

The BMPs play major roles in many key cellular areas, from cell proliferation to survival[15]. The BMPs have vital roles in maintaining tissue homeostasis during embryonic development. The BMP-2 has been shown to increase the proliferation of NP cells. If NP cells, which should divide steadily over their long, fixed lifespan, proliferate rapidly owing to prolonged rhBMP-2 treatment, the NP cells would be exhausted and become senescent, which would cause rapid IVDD[15].

In a study where it was reported that BMP-2 plays an important role in the healing process after disc injury, inflammation, chemotaxis and degeneration, it has been reported that this situation can be controlled by targeting BMP-2[10].

In the present study, BMP-2 protein expression increased by 54% in the primary cell cultures treated with TCZ compared with the control samples.

The Hif-1α plays a significant role by participating in genesis, energy metabolism and ECM synthesis[16]. Furthermore, Hif-1α can activate macroautophagy/autophagy through various pathways. The positive effects of Hif-1α on upregulating autophagy, decreasing apoptosis and promoting ECM synthesis in NP cells consequently retarded the progression of excessive mechanical loading-induced IVDD[16].

It has been emphasized in the literature that controlling hypoxia-related signalling pathways in disc degeneration may be a potential therapeutic option in the future. Under low oxygen conditions, Hif-1α initiates expression of its target genes, including proinflammatory cytokines and vascular endothelial growth factor, which is a family of growth factors associated with neovascularization[30].

In this study, HIF-1α protein expression increased by 28% in the primary cultures treated with TCZ compared with the control samples.

It is among the known facts that IL-1β significantly increases the apoptosis rate of NP cells. It has been reported that disc degeneration can be repaired by targeting BMP-2 and IL-1β[31].

Upregulation of IL-1β and downregulation of BMP-2 and SOX9 genes induce senescence in chondrocyte cells located in cartilage-like tissues[13]. The IL-1β, a proinflammatory cytokine, is associated with IVDD[20,32]. The IL-1β is frequently preferred in studies that examine IVDD because it induces apoptosis in NP cells[33].

Cellular senescence associated with inflammation plays an important role in the pathogenesis of many IVDD-related diseases, especially osteoarthritis[34]. High expression levels of proinflammatory cytokines such as IL-1β cause senescence, which could lead to pathological diseases[35]. In addition, many cytokines, especially IL-1β and transcription factors such as BMP-2 and SOX9 make important contributions to the process of chondrogenic differentiation[36]. The SOX9 is activated by the expression of TGF-β and BMP during very early events in chondrogenesis and, directly or indirectly, maintains their regulation during the differentiation and maturation of chondrocytes[36]. The SOX9 keeps NP growth healthy by inhibiting cell dedifferentiation or redifferentiation[37].

In this study, IL-1β protein expression increased by 304% in the primary cultures treated with TCZ compared with the control samples.

The effects of TCZ administered to human primary AF and NP cell cultures on IVD tissue and the ECM structure examined in this study were in line with the literature[33-37]. The study sought to determine the effect of TCZ on the senescence mechanisms associated with IVDD and/or inflammation and assessed the protein expression levels of BMP-2, Hif-1α, IL-1β and SOX9. The proliferation steadily decreased on days 1, 7 and 15 in the TCZ-treated samples. This decrease was statistically significant ($p<0.05$). The BMP-2, Hif-1α, SOX9 and IL-1β expressions increased by 54, 28, 99 and 304%, respectively, in the TCZ-treated samples compared with the untreated samples ($p<0.05$).

All culture samples were prepared using tissues obtained from patients who were of the same race, generating the first limitation of this research and this research was carried out in an *in vitro* experimental setup, so the compensatory mechanisms in the body were disabled.

CONCLUSION

The changes in protein expressions of BMP-2, Hif-1α, IL-1β and SOX9 observed after administration of TCZ to primary cell cultures isolated from disc tissues show promise in future target therapies for IVD inflammation and degeneration. It is important that protein expressions of BMP-2, Hif-1α and SOX9, which are important in the anabolism of disc degeneration, increased after TCZ application. In this way, TCZ, which will be manipulated by the pharmaceutical industry, may be an option for the regeneration of IVDD in the future. This study had an *in vitro* experimental design. However, the results of the present study could offer insights into degeneration, inflammation pathogenesis and the senescence mechanism of IVD and a gateway to future studies.

SIGNIFICANCE STATEMENT

The purpose of this study is to evaluate the effects of the immunosuppressant tocilizumab (TCZ), which also has a place in the treatment of covid-19, on the persistence of IVD cells and inflammation-related signalling pathways. In the study, it was determined that TCZ application decreased the proliferation of IVD cells and increased the expression of BMP-2, Hif-1α, SOX9 and IL-1β. Therefore, it can be argued

that drug administration may adversely affect the prognosis in IVDD cases. Of course, studies involving animal experiments and evaluating other signalling pathways related to IVD catabolism are needed to predict the effect in clinical applications.

REFERENCES

1. Marra, F., E.J. Smolders, O. El-Sherif, A. Boyle and K. Davidson *et al*., 2021. Recommendations for dosing of repurposed Covid-19 medications in patients with renal and hepatic impairment. Drugs RD, 21: 9-27.
2. Shuai, Z.Q., C.X. Zhang, Z.W. Shuai and S.L. Ge, 2021. Efficacy and safety of biological agents in the treatment of patients with Takayasu arteritis: A systematic review and meta-analysis. Eur. Rev. Med. Pharmacol. Sci., 25: 250-262.
3. Xu, X., M. Han, T. Li, W. Sun and D. Wang *et al*., 2020. Effective treatment of severe COVID-19 patients with tocilizumab. Proc. Natl. Acad. Sci. U.S.A., 117: 10970-10975.
4. del Pozo, J.S.G., M.F. Galindo, E. Nava and J. Jordán, 2020. A systematic review on the efficacy and safety of IL-6 modulatory drugs in the treatment of COVID-19 patients. Eur. Rev. Med. Pharmacol. Sci., 24: 7475-7484.
5. Bersanelli, M., 2020. Controversies about COVID-19 and anticancer treatment with immune checkpoint inhibitors. Immunotherapy, 12: 269-273.
6. Jamilloux, Y., T. Henry, A. Belot, S. Viel and M. Fauter *et al*., 2020. Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. Autoimmu. Rev., Vol. 19. 10.1016/j.autrev.2020.102567.
7. Wang, L., X. Peng, Z.-H. Wang, J. Cai and F.-C. Zhou, 2020. Tocilizumab in the treatment of a critical COVID-19 patient: A case report. Eur. Rev. Med. Pharmacol. Sci., 24: 5783-5787.
8. Coleman, J.J. and S.K. Pontefract, 2018. Adverse drug reaction. Clin. Med., 16: 481-485.
9. Yilmaz, I., H. Akalan, D.S. Yasar, N. Karaarslan, H. Ozbek and O. Ates, 2022. Is favipiravir a potential therapeutic agent in the treatment of intervertebral disc degeneration by suppressing autophagy and apoptosis? Turk. Neurosurg., 32: 680-687.
10. Hsu, Y.H., R.M. Lin, Y.S. Chiu, W.L. Liu and K.Y. Huang, 2020. Effects of IL-1β, IL-20, and BMP-2 on intervertebral disc inflammation under hypoxia. J. Clin. Med., Vol. 9. 10.3390/jcm9010140.
11. Caliskan, T., D.Y. Sirin, N. Karaarslan, I. Yilmaz and H. Ozbek, *et al*., 2019. Effects of etanercept, a tumor necrosis factor receptor fusion protein, on primary cell cultures prepared from intact human intervertebral disc tissue. Exp. Ther. Med., 18: 69-76.
12. Chiu, M.N., M. Bhardwaj and S.P. Sah, 2022. Safety profile of COVID-19 drugs in a real clinical setting. Eur. J. Clin. Pharmacol., 78: 733-753.
13. Ashraf, S., B.H. Cha, J.S. Kim, J. Ahn, I. Han, H. Park and S.H. Lee, 2016. Regulation of senescence associated signaling mechanisms in chondrocytes for cartilage tissue regeneration. Osteoarthritis Cartilage, 24: 196-205.
14. Tsingas, M., O.K. Ottone, A. Haseeb, R.A. Barve, I.M. Shapiro, V. Lefebvre and M.V. Risbud, 2020. *Sox9* deletion causes severe intervertebral disc degeneration characterized by apoptosis, matrix remodeling, and compartment-specific transcriptomic changes. Matrix Biol., 94: 110-133.
15. Kim, K.W., S.W. Jeong, H.Y. Park, J.Y. Heu, H.Y. Jung and J.S. Lee, 2020. The effect of prolonged rhBMP-2 treatment on telomerase activity, replicative capacity and senescence of human nucleus pulposus cells. Biotech. Histochem., 95: 490-498.
16. He, R., Z. Wang, M. Cui, S. Liu and W. Wu *et al*., 2021. HIF1A alleviates compression-induced apoptosis of nucleus pulposus derived stem cells via upregulating autophagy. Autophagy, 17: 3338-3360.
17. Zeng, Y., K.G. Danielson, T.J. Albert, I.M. Shapiro and M.V. Risbud, 2007. HIF-1α is a regulator of galectin-3 expression in the intervertebral disc. J. Bone Miner. Res., 22: 1851-1861.
18. Karaarslan, N., I. Yilmaz, H. Ozbek, D.Y. Sirin and N. Kaplan *et al*., 2019. Are radio-contrast agents commonly used in discography toxic to the intact intervertebral disc tissue cells? Basic Clin. Pharmacol. Toxicol., 124: 181-189.
19. Paccaly, A.J., P. Kovalenko, J. Parrino, A. Boyapati and C. Xu *et al*., 2021. Pharmacokinetics and pharmacodynamics of subcutaneous sarilumab and intravenous tocilizumab following single-dose administration in patients with active rheumatoid arthritis on stable methotrexate. J. Clin. Pharmacol., 61: 90-104.
20. Yilmaz, I., H. Akalan, D.Y. Sirin, N. Karaarslan, N. Kaplan and H. Ozbek, 2022. Effects of an acetylcholinesterase inhibitor and an N-methyl-D-aspartate receptor antagonist on inflammation and degeneration of the nucleus pulposus. Eur. Rev. Med. Pharmacol. Sci., 26 : 4409-4419.
21. Yilmaz, I., H. Akalan, N. Karaarslan, D.Y. Sirin and N. Kaplan *et al*., 2022. Can transcription factors in the intervertebral disc of lopinavir/ritonavir prevent degeneration in the nucleus pulposus by mediating the regulation of inflammation through signaling pathways? Eur. Rev. Med. Pharmacol. Sci., 26: 6845-6855.
22. Yilmaz, I. and N. Karaarslan, 2022. Examining the effects of HMG-CoA reductase inhibitors on anabolic and catabolic signaling pathway proteins associated with degenerative disc disease. Eur. Rev. Med. Pharmacol. Sci., 26 : 2990-3000.
23. Yilmaz, I., N. Karaarslan, D.Y. Sirin and H. Ozbek, 2020. Pharmaco-molecular assessment of the effects of anandamide and its antagonists on hippocampal tissue in Wistar albino rats. Eur. Rev. Med. Pharmacol. Sci., 24: 11871-11882.

24. Yilmaz, I., H. Akalan, K. Oznam, N. Karaarslan, D.Y. Sirin and H. Ozbek, 2022. Does oseltamivir protect human chondrocyte and nucleus pulposus cells from degeneration by inhibiting senescence and proinflammation mediated by the $NLRP_3$ inflammasome and NF-κB? Eur. Rev. Med. Pharmacol. Sci., 26: 4816-4827.
25. Wang, S., J. Zhou, W. Kang, Z. Dong and H. Wang, 2016. Tocilizumab inhibits neuronal cell apoptosis and activates STAT3 in cerebral infarction rat model. Bosn. J. Basic Med. Sci., 16: 145-50.
26. Karaarslan, N., I. Yilmaz and D.Y. Sirin, 2021. Toxicity of the acetyl-para-aminophenol group of medicines to intact intervertebral disc tissue cells. Exp. Ther. Med., Vol. 21. 10.3892/etm.2020.9578.
27. Du, J., M. Xu, F. Kong, P. Zhu and Y. Mao *et al*., 2022. CB2R attenuates intervertebral disc degeneration by delaying nucleus pulposus cell senescence through AMPK/GSK3β pathway. Aging Dis., 13: 552-567.
28. Zhang, S., S. Song, Y. Zhuang, J. Hu and W. Cui *et al*., 2021. RETRACTED: Role of microRNA-15a-5p/Sox9/NF-κB axis in inflammatory factors and apoptosis of murine nucleus pulposus cells in intervertebral disc degeneration. Life Sci.,Vol. 277. 10.1016/j.lfs.2021.119408.
29. Zhang, G.Z., M.Q. Liu, H.W. Chen, Z.L. Wu and Y.C. Gao, 2021. NF-κB signalling pathways in nucleus pulposus cell function and intervertebral disc degeneration. Cell Prolif., Vol. 54. 10.1111/cpr.13057.
30. Kwon, W.K., H.J. Moon, T.H. Kwon, Y.K. Park and J.H. Kim, 2017. The role of hypoxia in angiogenesis and extracellular matrix regulation of intervertebral disc cells during inflammatory reactions. Neurosurgery, 81: 867-875.
31. Ohnishi, T., N. Iwasaki and H. Sudo, 2022. Causes of and molecular targets for the treatment of intervertebral disc degeneration: A review. Cells, Vol. 11. 10.3390/cells11030394.
32. Zou, L., H. Lei, J. Shen, X. Liu and X. Zhang *et al*., 2020. HO-1 induced autophagy protects against IL-1 β-mediated apoptosis in human nucleus pulposus cells by inhibiting NF-κB. Aging, 12: 2440-2452.
33. Zhang, Y., F. He, Z. Chen, Q. Su and M. Yan *et al*., 2019. Melatonin modulates IL-1β-induced extracellular matrix remodeling in human nucleus pulposus cells and attenuates rat intervertebral disc degeneration and inflammation. Aging, 11: 10499-10512.
34. Chai, B., Z.H. Zheng, X. Liao, K.Y. Li and J.S. Liang *et al*., 2020. The protective role of omentin-1 in IL-1β-induced chondrocyte senescence. Artif. Cells Nanomed. Biotechnol., 48: 8-14.
35. Zhang, D., G. Zhang, Z. Li and B. Li, 2018. Activation of the cannabinoid receptor 1 by ACEA suppresses senescence in human primary chondrocytes through sirt1 activation. Exp. Biol. Med., 243: 437-443.
36. Huh, J.E., P.S. Koh, B.K. Seo, Y.C. Park, Y.H. Baek, J.D. Lee and D.S. Park, 2014. Mangiferin reduces the inhibition of chondrogenic differentiation by IL-1β in mesenchymal stem cells from subchondral bone and targets multiple aspects of the Smad and SOX9 pathways. Int. J. Mol. Sci., 15: 16025-16042.
37. Nukaga, T., D. Sakai, J. Schol, K. Suyama, T. Nakai, A. Hiyama and M. Watanabe, 2019. Minimal sustainability of dedifferentiation by ROCK inhibitor on rat nucleus pulposus cells *in vitro*. Spine Surg. Relat. Res., 3: 385-391.

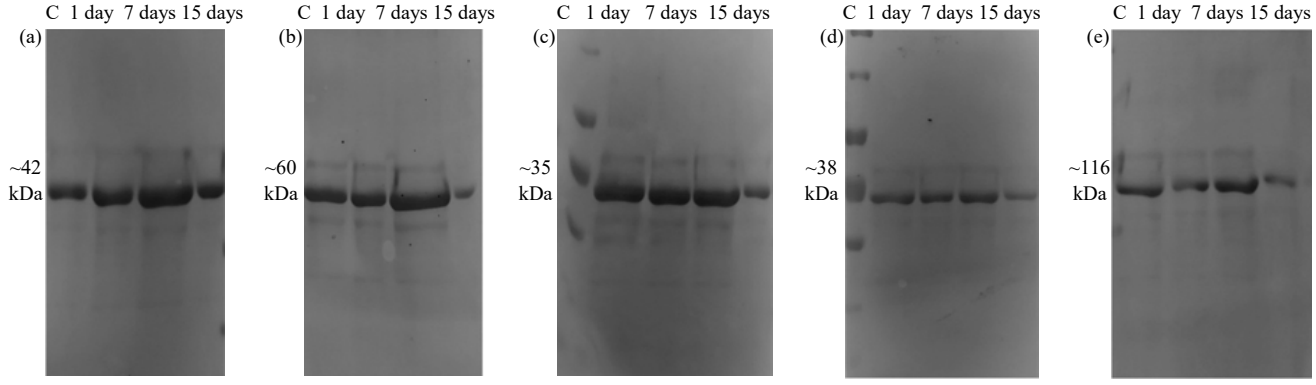

Fig. S4(a-e): Illustrative Western blots for all groups (a) β-actin, (b) SOX9, (c) IL-1β, (d) BMP-2 and (e) HIF-1α