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The effect of favipiravir on histone deacetylase (HDAC) enzyme activity in administered to human primary chondrocyte cultures and an evaluation of its relationship with inflammation

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ABSTRACT

Aim: The existing literature is inconclusive about the in vitro cytotoxicity of favipiravir (FVP) administered with valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, and the effects of FVP on HDAC activity and inflammation markers. This study aimed to evaluate the in vitro effects of FVP administered to human primary chondrocyte cultures by investigating its effects on HDAC enzyme activity and interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha (TNF-α) gene expression levels.

Methods: Primary chondrocyte cultures were prepared using tissues obtained from operated cases. Untreated primary cell culture samples constituted the control group. Cultures administered with FVP were the study group. Analyses were performed simultaneously on the samples in both groups at the end of 0, 24, 48, and 72 h. The surface morphology of the cells and the extracellular matrix were evaluated using an inverted light microscope. Cell viability, proliferation, and cytotoxicity evaluations were investigated using MTT analysis and acridine orange/propidium iodide stains. Commercial kits were used to measure HDAC enzyme activity and IL-6, IL-10, and TNF-α expression levels. An enzyme-linked immunosorbent assay was performed to evaluate the cell inflammatory responses. The data were analyzed statistically.

Results: FVP did not change the HDAC activity of the primary cultures; however, it produced a proinflammatory response by increasing levels of TNF-α in the cells and an anti-inflammatory response by increasing levels of IL-10.

Conclusions: FVP-induced proinflammatory responses in primary chondrocytes are independent of HDAC activity, with potential resistance to inflammatory responses through an additional anti-inflammatory response.

Key words: Chondrocytes, histone deacetylase, favipiravir, valproic acid, IL, TNF-α.

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1. Introduction

Most medications administered orally or parenterally accumulate in the synovial fluid compartment [1-4]. Medications and nutrients initially diffuse in significant quantities into

hyaluronan or synovial tissues before spreading into body fluids. They then pass through the pores in the hyaline membrane in the cartilage tissue to reach the cells. However, aneuronal cartilage tissue is alymphatic [5].

Favipiravir (FVP) is being used in many countries to treat the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection pandemic, also called COVID-19, which began to rapidly affect the entire world in 2019 and has caused significant public health concerns [6, 7]. In the existing literature, no studies with a high level of evidence have evaluated the effect of FVP on the proliferation of chondrocytes, which are cartilage tissue cells. There are no studies in the literature evaluating the impact of any positive or negative effect of FVP on the activation and inhibition of histone deacetylase (HDAC), which plays an important role in the epigenetic mechanisms of chondrocytes.

Chromatin and DNA modifications control gene expression at the transcriptional level. Many biological events, including proliferation, are controlled through various post-translational modifications occurring in the amino tails of histone structures [8]. One of the most important chromatin DNA modifications is histone acetylation [9]. The electrostatic charge of histones changes with acetylation. Thus, the binding of transcription factors to DNA is facilitated, and the affinity of histone for DNA decreases due to transcription activation [10]. The acetylation state of histones is controlled by a reversible equilibrium between histone acetyltransferase and HDAC enzyme families. There are medications targeting HDAC enzymes because they affect important cellular events, and compounds that can inhibit these enzymes have been identified and used in treatment protocols [11-13].

The HDAC enzyme family has excellent potential as a medication target, since it is associated with many biological events, such as cell cycle regulation, cell proliferation, survival, differentiation, metabolism, and DNA repair. Therefore, investigating the activation of HDAC enzymes has gained popularity [11-13]. HDAC inhibitors have been proposed for the treatment of numerous diseases, such as cancer, spinal muscular atrophy, Alzheimer's disease, Parkinson's disease, and diabetes [11-13]. Many studies have argued that HDAC regulates the stability and activity of hypoxia-induced factor-1 alpha (HIF-1α) protein in nucleus pulposus cells [14-16]. However, its effect on chondrocytes has not been fully revealed.

Valproic acid (VPA), which is used in clinical practice for the treatment of bipolar and mood disorders, as well as epilepsy, inhibits HDAC activity in vitro and in vivo and leads to histone [17]. Animal studies have shown that this anti-epileptic drug has protective effects in several tissues *via* anti-inflammatory, anti-apoptotic, and anti-fibrotic properties [18]. VPA protects against inflammation and apoptosis, and these effects are independent of inhibition. VPA has anti-inflammatory effects, is mediated by matrix metalloproteinase-9 inhibition, and reduces inflammatory cytokine levels [19]. VPA inhibits the induction of specific cytokines/chemokines [20].

Interleukin-6 (IL-6) is a pro-inflammatory cytokine involved in the pathophysiology of several age-related diseases, such as osteoporosis. IL-6 can induce pro-inflammatory mediators and extracellular matrix-degrading enzymes, causing cartilage degeneration. Experimental osteoarthritis models have demonstrated that blocking IL-6 can protect against cartilage degradation [21]. Similarly, an increase in the expression of TNF-α, a pro-

inflammatory cytokine, is observed in osteoporosis patients and causes cartilage cell death [22]. IL-10 is an anti-inflammatory cytokine involved in suppressing inflammation of the synovial membrane, slowing the progression of osteoarthritis (OA), and promoting cartilage turnover [23].

The Science Committee of the Ministry of Health in Turkey has established a treatment protocol to manage SARS-CoV2 patients. In accordance with the treatment protocol, the patients are administered a loading dose of 2 × 1600 mg (200 mg tablet) favipiravir on the first day, followed by 600 mg orally twice daily for four days [24]. This study aims to investigate whether FVP, which is frequently used in the treatment of COVID-19 against SARS-Cov-2 infection, has a toxic effect on cartilage tissue cells and the extracellular matrix (ECM). Furthermore, we investigated changes in HDAC enzyme activity in FVP-treated cultures and evaluated potential inflammatory responses in the cells by assessing the gene expression levels of IL-6, IL10, and TNF-α.

2. Materials and metods

All experiments were repeated at least three times to minimize errors. The same surgical team prepared the osteochondral tissues used in the primary cultures. The researchers who conducted the molecular and histopathological analyzes in the laboratory did not have information regarding which cell culture samples were medicated or which medications the cultures included; thus, they were blind to the research. All experiments were performed simultaneously 0, 24, 48, and 72 h after administering the medication. Each experiment was performed at least three times.

2.1. Research inclusion or exclusion criteria of cases whose tissues were used in preparing the primary cell cultures: The tissues used in preparing the primary chondrocyte cultures were obtained from patients classified as grade 4 on the Kellgren–Lawrence radiological scale (n = 8) [1-5]. The intact and healthy parts of the resected tissues were used in the culture phase. The cartilage tissue samples were extracted from primary (idiopathic) osteoarthritis patients who were of normal weight (body mass index: 18.5–24.9) and who did not have any additional chronic diseases that required regular drug use. The patients ranged in age from 60 to 75 years. The study excluded patients who had experienced osteoarthritis due to trauma, inflammatory arthritis, or septic arthritis and those who had undergone previous surgery on the same knee. The study included patients with primary (idiopathic) gonarthrosis. The cartilage tissue samples were obtained from femoral and tibial bones during the "total knee arthroplasty surgery" of eight patients (four female, four male) with primary gonarthrosis whose operated knees were grade 4 according to the Kellgren-Lawrence grading system and who had knee pain unresponsive to conservative treatments. Of these bone-cartilage tissue remnants obtained during surgery, the tissues located in the parts of the knee joint that were less exposed to load and where osteoarthritis findings were less or not seen were preferred. The cartilage tissues were obtained from parts that appeared morphologically healthier than their surroundings and were evaluated as stage 0 (normal cartilage) according to the Outerbridge classification [25].

2.2. Dissection of the tissues by surgical resection and preparation of primary cell cultures: The cartilage and bone tissues of these osteochondral tissues were separated with a scalpel. The cartilage tissues were placed in sterile containers and were the only tissues used in this study [1-5]. The tissues were transferred to Falcon tubes containing medium and

penicillin-streptomycin and transferred to the laboratory at 4°C under aseptic conditions. Tissues were degraded in separate petri dishes, first mechanically with a rongeur and then enzymatically with the help of 200 units/mL collagenase type II enzyme dissolved in Hank's balanced salt solution. The samples were incubated overnight in an incubator with a 5% CO_2 medium. The tissues were later centrifuged at 4°C and 1200 rpm twice consecutively for 10 min. The supernatant was removed, a freshly prepared cell culture medium was added to the pellets that settled at the bottom of the tubes, and the cell groups in the pellets were resuspended. The samples were then transferred to the flasks separately [1-5]. Cells that were living and attached to the surface of the flasks were removed and resuspended by treatment with trypsin. The number of living cells was determined by counting in the presence of trypan blue on a Neubauer slide. A suspension of the counted cells was seeded into cultures on 96-well plates at 10000 cell/well for MTT analyses and acridine orange/propidium iodide stains (AO/PI) stains and into 24-well plates at 2.1×10^5 cell/well to perform HDAC activity assay and IL-6, IL-10, and TNF-α ELISA experiments. These cultures were incubated for 24 h at 37°C in an incubator containing 5% CO_2 before treatment with the drugs.

2.3. Administration of medications to primary cultures: The plasma half-life of FVP is nearly 4 h [1]. In clinical practice, FVP is administered orally for a total of five days, with a 2 × 1600 mg one-day loading dose followed by maintenance doses of 2 × 300 mg for four days [1, 24]. Previous studies have mimicked these clinical doses in cell cultures [26, 27]. In this study, FVP was dissolved in a growth medium containing 0.5% DMSO, and a stock solution with a 4 mg/ml concentration was prepared. This stock solution was diluted with the medium and applied to chondrocyte cultures with a final concentration of 62 µM [28]. A stock solution of 10 mg/ml concentration was also prepared by dissolving VPA in a medium containing 0.5% DMSO. This stock solution was diluted with the medium and applied to chondrocyte cultures with a final concentration of 0.5 mM [29]. In the present study, FVP and VPA doses were used, following previous studies. The present study did not confirm the toxic doses revealed in previous studies. Our aim was to reveal whether these drugs have effects on HDAC activity and inflammation, even at doses that may not have toxic effects on cells in vitro. The control group was not treated with the drugs and was classified as Group 1. Group 2 samples received 0.5 nM of VPA but no FVP. Group 3 samples were treated with 62 µM of FVP but no VPA.

2.4. Histopathological evaluations using inverted light microscopy with light and fluorescent attachments: The surface morphologies of the cells and the ECM were evaluated using inverted light microscopy at ×4, ×10, ×20, and ×40 magnifications. To better evaluate cell morphology, a Giemsa stain was also performed, and micrographs were obtained. AO/PI staining was performed in all cultures and displayed with the help of a fluorescent microscope under similar magnifications. The results obtained from the AO/PI stains enabled the separation of live and dead cells, as live cells glow green while dead cells appear red. The data obtained from the MTT analyses were confirmed using this method.

2.5. 3-(4,5-Dimethylthiazol2-yl)-2,5-diphenyltetrazolium-bromide (MTT) cell viability, toxicity, and proliferation test: A Vybrant MTT Cell Proliferation Assay commercial kit (Cat#V13154, Thermo Fisher Scientific, Waltham, MA, USA) was used to measure cell viability and toxicity. The MTT analyses were performed at 540 nm absorbance.

Here, the viability of the cells in the control group samples, which were not initially treated with FVP, was accepted as 100%. Cell proliferation was calculated using *Test OD/ [Control OD])* × *100* and the proliferation inhibition was calculated using *1-Test OD/Control OD*. The data were recorded for statistical analysis [1-5].

2.6. HDAC activity assay: A Biovision brand HDAC Activity Colorimetric Assay Kit (catalog no: K331-100) was used to evaluate whether FVP administration had an effect on HDAC activity. The experimental procedures followed the kit protocol. Briefly, culture supernatants (i.e., the culture medium) were collected after the drug administration at the specified times in all experimental and control groups. These supernatants were stored at -20°C for later assessment of IL-6, IL-10, and TNF-α levels. The cells attached to the culture dish were washed once with 1× phosphate buffer solution, and the medium was completely removed. Next, NETN buffer (prepared as 0.5 M EDTA, pH 8.0 with a final concentration of 5 mM and 1 M Tris-HCl, pH 8.0 with a final concentration of 50 mM and 0.5% NP-40) containing a protease inhibitor cocktail (Abcam, ab65621) was added to lyse the cells. Cell debris was precipitated by centrifugation, and the experiment was continued with the supernatant containing the nuclear extract. The amount of total protein in the supernatant containing nuclear extract was determined with the Bradford method, and all samples were diluted with ddH_2O to contain 300 µg of protein. Next, 85 µL of each sample extract was applied to the wells of an ELISA plate. Then, 10 µL of 10X HDAC assay buffer and 5 µL of HDAC colorimetric substrate were added to each well, and the wells were incubated at 37°C for 1 h. The reaction was terminated by adding 10 µL of lysine developer and incubating the plate at 37°C for 30 min. The OD values were then obtained using an ELISA microplate reader at 400 nm absorbance. In this experiment, a HeLa nuclear extract in the kit was used as the positive control to reveal the presence of HDAC activity or that the experiment was running, and trichostatin, an HDAC inhibitor included in the kit, was used as the negative control.

BOSTER commercial kits (IL-6, catalog no: EK0410; IL-10, catalog no: EK0416; TNF-α, catalog no: EK0525) were used to determine the amounts of IL-6, IL-10, and TNF-α. Protein amount was primarily determined in the culture supernatants. For IL-6, IL-10, and TNF-α, standard curves were first created for each protein using the standards included in the kit. Thus, the IL-6, IL-10, and TNF-α amounts in the samples were calculated as the percentage of the total protein, and the differences between the groups were determined. Subsequent steps for IL-6, IL-10, and TNF-α were in accordance with the kit protocol. A 100-µL sample was added to the microplate strips in the kit, and the plate was incubated at 37°C for 90 min. The plate was then emptied, and 100 µL of 1× biotinylated anti-human IL-6, IL-10, or TNF-α was added, and the mixture was incubated at 37°C for 60 min. The mixture was removed, and the wells were washed three times with 1× wash buffer, and 100 µL of 1× avidin biotin peroxidase complex was added and incubated at 37°C for 30 min. The wells were washed five times before adding a 90 µL color-developing reagent and incubating for 25 min at 37°C. Lastly, 100 µL of stop solution was added to each well, and OD values on an ELISA microplate reader at 450 nm absorbance.

2.7. Statistical analyses: The Minitab (Version 22.0) statistical package program was used to evaluate the data. Variables were presented as mean ± standard deviation, percentage (%), or fold. A factorial analysis of variance was used to compare the mean values.

Multiple comparisons were performed with Tukey's HSD test. A value of $p < 0.05$ was accepted as significant.

3. Results

3.1. Demographic data: The mean age of the cases (n = 8; four males, four females) whose tissues were used in the preparation of primary cartilage tissue cell cultures was 56.12 ± 2.71 years.

3.2. Microscopic evaluations: At 0, 24, 48, and 72 h, the cells were evaluated morphologically in all experimental and control groups and visualized with the help of an inverted microscope (Figure 1).

The administration of FVP or VPA at these doses did not affect the chondrocyte morphology, and the cells continued to proliferate. Figures 1A and 1B show the unstained and Giemsa-stained inverted microscopic images, respectively, of the control samples at 0 h, while Figures 1D and 1E are the experimental groups treated with FVP for 24 h. Figures 1J and 1K show the results for the 48-h samples, and Figures 1P and 1Q show the cells at 72 h. Figures 1G and 1H show the unstained and Giemsa-stained inverted microscopic images of the samples, respectively, for the experimental groups in which FVP was applied for 24 h, while Figures 1M and 1N show the results for 48 h. Figures 1S and 1T show the results for 72 h. Figure 1C, 1F, 1L, and 1R show the AO/PI staining images of VPA-treated cultures at 0, 24, 48, and 72 h, respectively. Figures 1I, 1O, and 1U show the AO/PI staining images of FVP-treated cultures at 24, 48, and 72 h, respectively. No cell death was observed in the cultures.

3.3. Evaluation of cell viability and toxicity: MTT analyses were performed on both experimental and control samples after 0, 24, 48, and 72 h of treatment. The viability of the control group, which was not incubated for the same duration, was accepted as 100%, and the

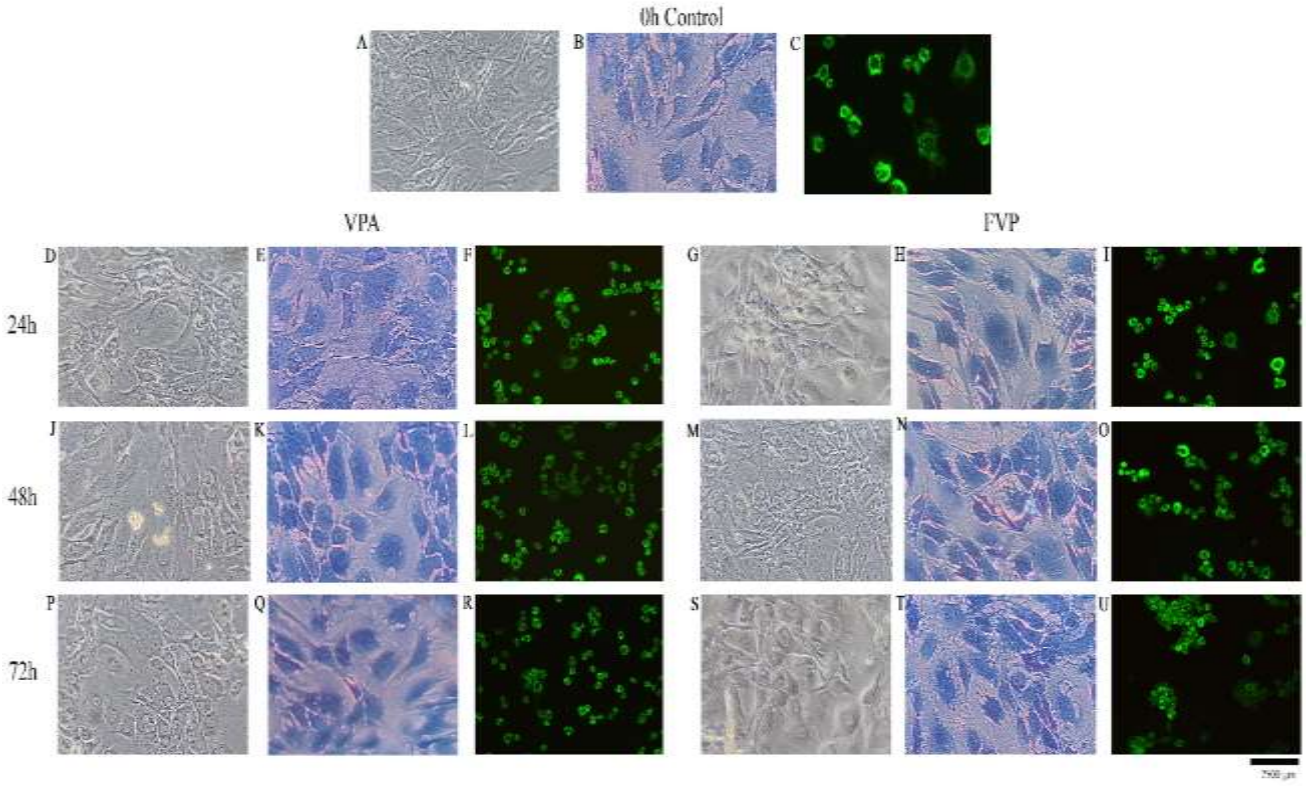

Figure 1. Morphological evaluation of the chondrocyte cultures.

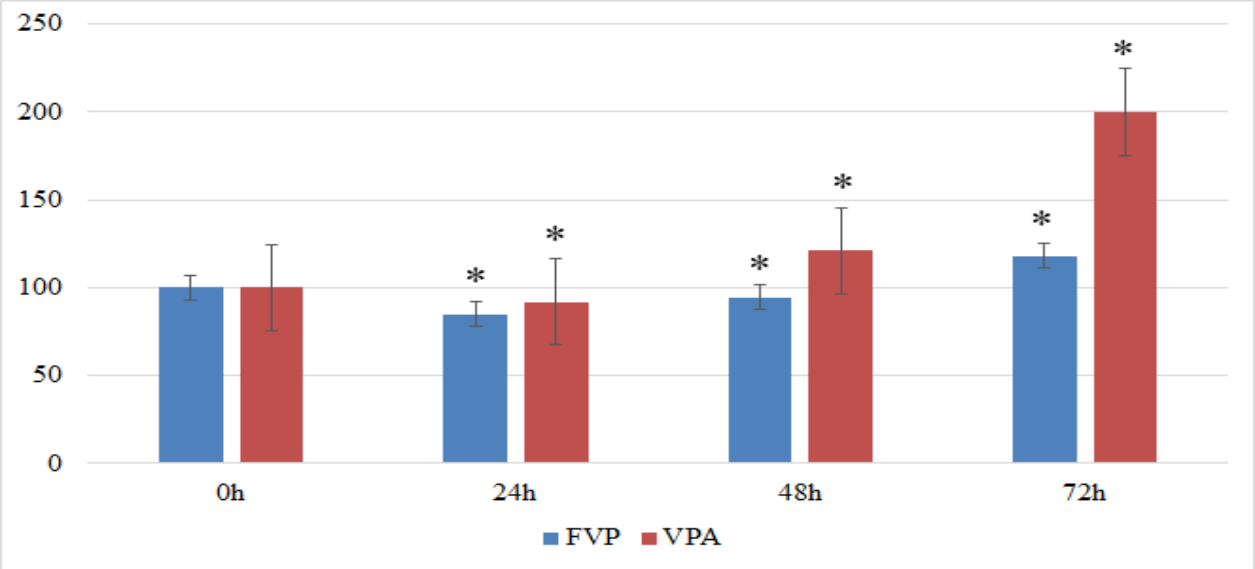

Figure 2. Evaluation of proliferation: Percentage of viability calculated in FVP- and VPA-treated cultures.

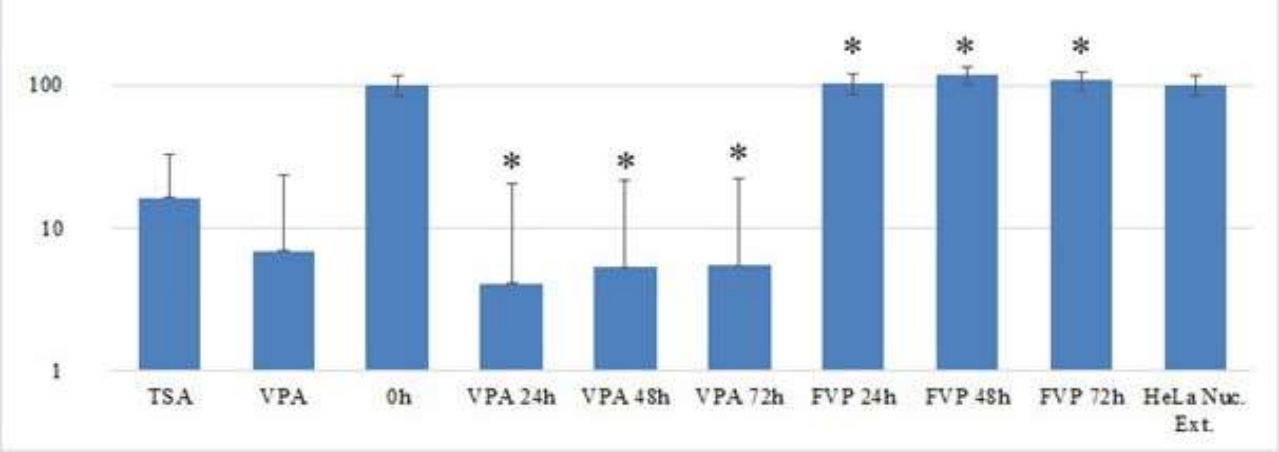

Figure 3. Graph of HDAC activity percentage: 0-h control group (HDAC activity 100%) vs. FVP- or VPA-treated cultures.

percentage of viability was calculated in the experimental groups. Accordingly, the viability percentage in the cultures treated with FVP was 85% at 24 h, 94.31% at 48 h, and 118.18% at 72 h. In the chondrocyte cultures treated with VPA, the viability percentage was found to be 91.25% at 24 h, 124.5% at 48 h, and 200% at 72 h (Figure 2).

These data reveal**ed** that administration of FVP or VPA **did** not affect cell viability at the administered doses and that the cells continu**ed** to proliferate. All these results were statistically significant ($p < 0.05$).

3.4. Evaluation of HDAC enzyme activation / inhibition: HDAC activity in 0-h control lysates was suppressed by 83.67% following treatment with trichostatin, an HDAC inhibitor included in the kit. This was taken as the full HDAC activity of 100% in this study. Thus, in the experimental groups treated with VPA, the relative suppression of HDAC activity compared to the 0-h control lysate was 93.09%. These two results constituted the technical negative controls. Furthermore, HDAC activity was found to be 100% in the HeLa nuclear extracts included in the kit, which constituted the technical positive control. In this vein, HDAC activity was suppressed by 95.90% in cultures treated with VPA for 24 h, 94.68% in cultures treated with VPA for 48 h, and 94.58% in cultures treated

with VPA for 72 h. FVP administration did not affect HDAC activity. In the cultures treated with FVP, HDAC activity was 104.03% at 24 h, 119.14% at 48 h, and 108.24% at 72 h. Figure 3 shows the HDAC activity percentage (Figure 3).

3.5. Results of IL-6, IL-10, and TNF-α ELISA: The IL-6, IL-10, and TNF-α levels of the 0-h control group were accepted as 100%, and the corresponding proportions of these cytokines in the experimental samples were calculated relative to this value. Accordingly, in the cells treated with VPA, IL-6 expression was found to be 100.90% at 24 h, 70.85% at 48 h, and 54.22% at 72 h; IL-10 expression was found to be 125% at 24 h, 91.27% at 48 h, and 80% at 72 h; and TNF-α expression was found to be 142.86% at 24 h, 163.27% at 48 h, and 80% at 72 h (Figure 4).

expression was found to be 106.48% at 24 h, 73.33% at 48 h, and 72.92% at 72 h; and TNF-α expression was found to be 79.37% at 24 h, 137.14% at 48 h, and 35.71% at 72 h. Furthermore, these results were statistically significant ($p < 0.05$).

4. Discussion

The in vitro effect of FVP against SARS-COV-1 and MERS-CoV viruses has been shown [6]. However, while there are many studies related to FVP, there are no studies investigating the cytotoxic effects of this medication in primary chondrocyte cultures and its effect on HDAC activity. More importantly, no study has provided information concerning the effect of FVP on proinflammatory IL-6, TNF-α, and anti-

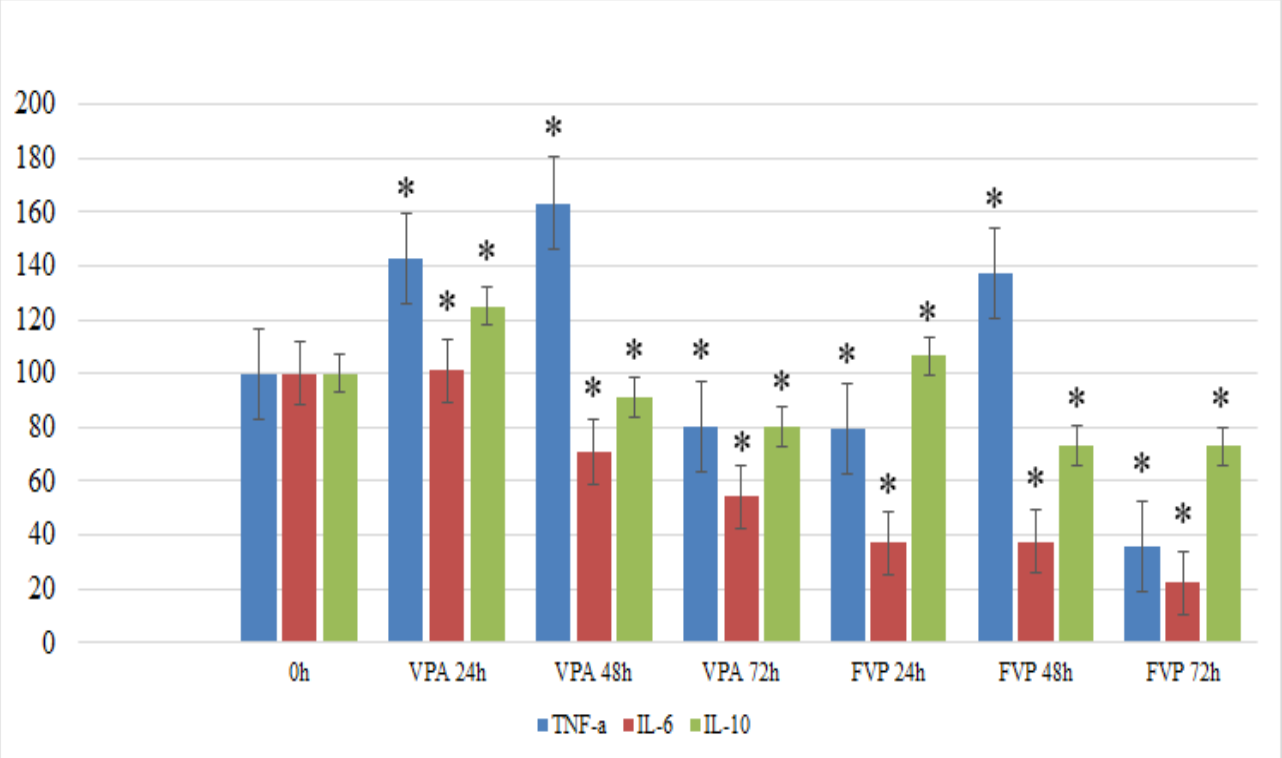

Figure 4. IL-6, IL-10, and TNF-α ELISA: Graph shows the percentage of IL-6, IL-10, and TNF-α; 0-h control group (100%) vs. FVP- or VPA-treated cultures.

In the cultures treated with FVP, IL-6 expression was found to be 37.15% at 24 h, 37.59% at 48 h, and 22.09% at 72 h; IL-10

inflammatory IL-10 expression in cartilage tissue and its effect on HDAC activity. Therefore, the data obtained from this study make significant

contributions to the literature.

This study aimed to determine whether the administration of FVP had a cytotoxic effect on chondrocytes. The morphological evaluations, AO/PI staining, and MTT analysis results were consistent, indicating that FVP administration did not cause cytotoxicity at the applied doses and durations. In the presence of the medication, the chondrocyte protected their viability and continued to proliferate. The study also aimed to determine whether FVP administration caused inflammation in chondrocytes and, if there was inflammation, whether its mechanism was related to HDAC activity.

HDAC activity is indispensable for controlling many biological events, such as DNA packaging, replication, repair, and gene expression [30]. Compounds that can inhibit HDAC enzymes have been shown to be effective in the suppression of cell division, the induction of apoptosis, differentiation, and other biological activities. Loss of acetylation condenses chromatin structure and suppresses gene expression. Studies have revealed a direct relationship between DNA methylation and histone modifications [31]. Currently, HDACs are classified into four families (classes I, II, III, and IV) according to their function, structure, distribution, and expression patterns. Many studies have reported the varying roles of different HDAC family members. For example, expression levels of HDAC 1 (HDAC1) are reduced in hMSCs chondrogenesis, and HDAC1 inhibition can elevate cartilage-specific gene expression and promote hMSCs chondrogenesis [32]. However, HDAC6 inhibition by TubA plays a valuable role in maintaining chondrocyte survival and preventing ECM degradation [33]. In the present study, a specific HDAC family member was not targeted. Instead, we evaluated the total HDAC activity in the primary chondrocyte cultures and found that the administration of VPA, an HDAC inhibitor, suppressed HDAC activity in these cells, whereas treatment with FVP changed HDAC activity in the cultures.

Numerous stress-related stimuli, including proinflammatory cytokines, can trigger cartilage tissue pathogenesis. Morphological changes observed in OA include cartilage erosion and varying degrees of synovial inflammation. Studies associate these changes with a complex network of biochemical factors, including proteolytic enzymes that lead to the degradation of cartilage macromolecules. Cytokines, such as IL-6 and TNF-α, which are produced by activated synoviocytes, mononuclear cells, or the articular cartilage itself, significantly increase matrix metalloproteinase gene expression. Cytokines also blunt the chondrocyte compensatory synthesis pathways needed to restore impaired ECM integrity [34].

Human chondrocytes synthesize IL-10 and express IL-10R on their surfaces. Since IL-10 inhibits the expression of IL-1 and TNF-α, the increase in IL10 in osteoarthritic chondrocytes may protect against the harmful effects of these catabolic cytokines. Furthermore, the functions of IL-10 in cartilage may go beyond the activities initiated in the immunological environment. High levels of IL-10 and IL-10R in fetal cartilage, which is an actively growing tissue, suggest that IL-10 may play a role in controlling chondrocyte metabolism under physiological conditions. IL-10 has been reported to restrict cartilage damage caused by blood present in the environment, particularly as a result of trauma or surgery [35, 36].

In the present study, the levels of TNF-α increased 42% at 24 h and 63% at 48 h in the chondrocytes treated with VPA, and total HDAC activity was suppressed by approximately 95%. However, the amount of TNF-α decreased 20% at 72 h compared to the control. Although the

level of IL-6 in the VPA-treated group at 24 h was the same as that of the control group, it continued to decrease at 48 and 72 h. IL-6 decreased 29.15% at 48 h and 45.78% at 72 h. In the same groups, the IL-10 level increased 25% at 24 h; however, compared to the control, it decreased by 8.73% at 48 h and 20% at 72 h. These results suggest that chondrocytes may respond rapidly to the increased TNF-α response in the first 48 h due to HDAC inhibition in these cultures, with the maintenance of viability and proliferation sustained by increasing the amount of IL-10. No HDAC inhibition was observed in the FVP-treated cultures.

Cell viability and proliferation in these cultures were weaker than in the VPA-treated cultures but were still maintained. FVP administration decreased TNF-α expression by 20.63% at 24 h in chondrocyte cultures; however, it increased by 37.14% at 48 h. At 72 h, the amount of TNF-α was 64.29% less than the control. In these cultures, an increase of 6.48% at 24 h was observed in IL-10. However, the amount of IL-10 decreased by 26.67% and 27.08% at 48 h and 72 h, respectively. This result may indicate that chondrocyte continuity can be achieved with increased IL-10 expression in response to increased TNF-α levels. Cartilage-specific extracellular matrix synthesis is a precondition for chondrocyte survival and cartilage function; however, it is affected by the pro-inflammatory cytokine TNF-α in arthritis. Overexpression of IL-10 differentially affects cartilage matrix gene expression in response to TNF-α in human joint chondrocytes in vitro. The overexpression of IL-10 is known to modulate some catabolic properties of TNF-α in chondrocytes [2]. Notably, there have been reports of the potential role of favipiravir in cell death by increasing necroptosis instead of apoptosis after the onset of inflammation [37, 38]. Thus, the present in vitro study provides convincing support for previous evidence of FVP mechanisms in the literature.

Another significant result of FVP administration was a dramatic decrease in IL-6 expression, which decreased 62.85% at 24 h, 62.41% at 48 h, and 77.91% at 72 h. FVP is also known to reduce IL-6 expression, particularly in viral infections. The present study has proven that this effect is also valid for cartilage cells. FVP is a broad-spectrum antiviral drug that targets RNA-dependent RNA polymerase [39]. Research evaluating the effects of FVP on HDAC activity is scant because the drug is relatively new. Therefore, the results from the present study present initial evidence of the effects of FVP on cartilage cell viability, HDAC activity, and inflammatory response and are highly valuable.

4.1. Conclusion

Our data reveal that the administration of FVP or VPA did not affect cell viability at the administered doses and that the cells continued to proliferate in the presence of these agents. Moreover, FVP administration did not affect HDAC activity. Although HDAC was not inhibited in chondrocyte cultures, IL-6, IL-10, and TNF-α expression were altered. Especially at 72 h, the decrease in these cytokines and TNF-α expression was significant. Although we cannot infer from these data that FVP application alone can increase inflammation in chondrocytes or decrease healing, we recommend careful evaluation of the dose and duration of application. As this was an in vitro study in primary cultures, follow-up studies should investigate our findings in more cases.

Abbreviations

AO/PI: acridine orange/propidium iodide stains; ECM: extracellular matrix; FVP: favipiravir; HDAC: histone deacetylase; IL: interleukin; SARS-CoV-2: severe acute respiratory

syndrome coronavirus 2; TNF-α: tumor necrosis factor-alpha; VPA: valproic acid

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