FLUORIDE

Quarterly Journal of The International Society for Fluoride Research Inc.

The Inflammatory Response of Human Fibroblast-Like Synoviocytes to Fluoride

Unique digital address (Digital object identifier [DOI] equivalent):
https://www.fluorideresearch.online/epub/files/346.pdf

Kamila Rerich[1], Maciej Tarnowski[2], Dariusz Chlubek[1*]

[1]Dr. Kamila Rerich, Department of Biochemistry and Medical Chemistry, Pomeranian Medical University in Szczecin, Poland
[2]Prof. Dr. Maciej Tarnowski, Department of Physiology in Health Sciences, Pomeranian Medical University in Szczecin, Poland
[1]Prof. Dr. Dariusz Chlubek, Department of Biochemistry and Medical Chemistry, Pomeranian Medical University in Szczecin, Poland

***Corresponding author:**
Prof. Dariusz Chlubek MD, PhD
Department of Biochemistry and Medical Chemistry
Pomeranian Medical University in Szczecin
Powstańców Wlkp. 72
70-111 Szczecin, Poland
Phone: (+48) 914661515
E-mail: dchlubek@pum.edu.pl

Accepted: 2025 Apr 13
Published as e346: 2025 Apr 15

ABSTRACT

Purpose: Chronic exposure to fluoride leads to the development of conditions resembling arthritis, which can be treated as functional disorders or diagnosed as arthritis. The pathogenesis of joint diseases related to fluoride toxicity is unclear. In this study, we hypothesized that fluoride affects fibroblast-like synoviocytes (FLS), one of the main populations of synovial cells present in the joint. FLS play a central role as mediators of joint damage in arthritis, as they produce cytokines and chemokines that recruit and activate leukocytes, as well as matrix metalloproteinases (MMPs) that degrade extracellular matrix proteins. Another characteristic of arthritis is the excessive proliferation and reduced susceptibility of FLS to apoptosis. Therefore, our study aimed to evaluate the effect of sodium fluoride (NaF) on the proliferation, apoptosis, and expression of pro-inflammatory cytokines and metalloproteinases in a human fibroblast-like synoviocyte (HFLS) cell line.

Methods: HFLS were exposed to NaF at concentrations corresponding to fluoride levels in the blood plasma of individuals living in non-endemic regions (1-2.5 µM) and individuals exposed to environmental fluoride (5-10 µM). HFLS proliferation and apoptosis were assessed by flow cytometry. mRNA expression of TNF-α, IL-6, IL-8, MMP-3, and MMP-9 was evaluated using real-time PCR. To our knowledge, this is the first study investigating the effects of NaF on HFLS.

Results: We did not find evidence that fluoride in the concentrations used stimulates excessive proliferation or limits apoptosis in HFLS. However, we observed increased expression of the pro-inflammatory cytokines TNF-α, IL-6, IL-8, and metalloproteinases MMP-3 and MMP-9.

Conclusions: We concluded that fluoride at concentrations of 5-10 µM initiates an inflammatory response in HFLS, which may be the beginning of joint inflammation and destruction.

Key-words: *Fluoride, Fibroblast-like Synoviocytes, Joint inflammation, Cytokines, Chemokines, Metalloproteinases*

INTRODUCTION

The prolonged high fluoride exposure results in development of arthritis-like syndromes, and finally chronic skeletal fluorosis. This problem applies particularly to residents of endemic regions where groundwater is rich in fluoride[1] and professionally exposed individuals.[2] The common initial symptoms of

fluoride toxicity in skeletal system are chronic fatigue, vague, diffuse aches, stiffness of joints with decreased range of motion and muscle weakness.[1] These symptoms may be dismissed as functional or arthritis (rheumatoid arthritis (RA), osteoarthritis (OA) or seronegative spondyloarthropathy), but in fact they may be early signs of fluoride poisoning and its damaging effect on tendinous insertions and ligaments as well as joint capsules.[3-9] During disease progression the bones undergo calcification (essentially spine), osteoporosis develops in long bones, what finally leads to weakness and painful of bones and joints, and disability.[1] A wide range of fluoride intoxication signs in musculoskeletal system have been described in the endemic areas, especially some regions of Asia[6,10-14] and Africa.[15,16]

The pathogenesis of joints disease associated with fluoride intoxication is not clear. Until now, the pathomechanism of bone lesions in skeletal fluorosis has been studied. It includes the process of bone turnover through impact of fluoride on osteoblasts and osteoclasts functions.[17,18] It is interesting whether the pathological processes also occur in the synovium like in rheumatic diseases. The direct fluoride effects on main cells of synovium, macrophage-like synoviocytes (MLS) and fibroblast-like synoviocytes (FLS), and their role in development of degenerative changes of joints is not investigated. Both types of synovium cells are known for the regulation of joint homeostasis, but also their participation in the pathogenesis of arthritis.[19] Previous studies indicated that sodium fluoride (NaF) (3-10 µM) promoted oxidative stress[20] and changed the amount and activity of enzymes, such as cyclooxygenases[21], 15-lipoxygenase[22] or phospholipase-2[23], in macrophages derived from THP-1 cells or peripheral blood mononuclear cells (PBMC). In this way, it contributes to the initiating and development of inflammatory process by macrophages.

We suspected that fluoride influences on the second main cellular component of the synovium, FLS, by changing their phenotype from normal to inflammatory. FLSs, together with MLS, form the two- or three-layer lining of joint synovium, which contacts with the intra-articular cavity and produces lubricious synovial fluid. FLSs, also known as type B synoviocytes, are mesenchymal cells that express specific adhesion molecules (integrins, ICAM-1, cadherin-11) and produce extracellular matrix proteins (type IV and V collagens) and components of synovial fluid (hyaluronan and lubricin), thanks to which these cells play structural and functional roles in the joints.[24,25] As a result of inflammation in the synovium, the normal lining structure is converted into a pannus-like structure, a hyperplastic synovial lining containing a higher number of activated FLSs and macrophages that spreads into the joint space, adhere to the cartilage surface, and attack and degrade the cartilage matrix causing joint destruction. What is important, FLSs are known as central mediator of join damage in arthritides because produce cytokines and chemokines that recruit and activate leukocytes, and matrix metalloproteinases (MMPs) that degrade extracellular matrix proteins.[23,24]

Hence, the present study was undertaken to evaluate the effects of NaF on proliferation of HFLS as well as the expression of pro-inflammatory cytokines and MMPs associated with the pathogenesis of arthritis.

MATERIAL AND METHODS

Reagents

All-in-one ready-to-use synoviocyte growth medium (SGM) and synoviocyte basal medium (SBM) were purchased from Cell Applications (San Diego, CA, USA). Trypsin-EDTA solution, trypsin inhibitor from Glycine max, Hanks' Balanced Salt solution, phosphate buffered saline (PBS) and fluoride ion solution for ISE (0,1 M NaF) was obtained from Sigma Aldrich (St. Louis, MO, USA). Fetal bovine serum was from Thermo Fisher Scientific (Gibco, Brasil).

Cell culture

The study was conducted on human fibroblast-like synoviocytes (HFLS), which were derived from normal synovial tissue and purchased as frozen vial from Cell Applications (San Diego, CA, USA) (catalog no. 408-05a). Cells were handled as recommended by the procedure and seeded in SGM (Cell Applications, San Diego, CA, USA) in 75 cm^2 culture flask (Sigma Aldrich, St. Louis, MO, USA). The flask was placed in humidified incubator at 37°C in an atmosphere 5% CO_2. The cells were subcultured when the HFLS culture reaches 80% confluency. HFLS were used to experiments between three and seven passages.

Proliferation assay

The HFLS were seeded at 2.0 x 10^3 cells per well in 96-wells plate with 100 µL SGM. The cells were cultured for 24 hrs and then medium were changed on SGM with (1-10 µM) or without sodium fluoride (control). Medium was changed every 24 hrs. The cell proliferation was evaluated after 24, 48 and 72 hrs culture by WST-1 cell proliferation assay (Roche, Basel, Switzerland). The absorbance of the formazan product was measured after 2 hrs incubation with 10 µL WST-1 on automated Microplate Reader EnVision 2104

(Perkin Elmer, Waltham, MA, USA) at 450 nm with correction at 620 nm.

Apoptosis assay

The HFLS were seeded into 6-well plates at an initial density of 12 × 10^4/mL SGM. The cells were cultured for 24 hours and then medium were changed on SGM with (1-10 µM) or without sodium fluoride (control). Apoptosis was evaluated after 24, 48 and 72 hours by the Annexin V-FITC Apoptosis Kit (BD Bioscience, San Diego, CA, USA). The harvested cells were washed with PBS, resuspended in 100 µL of buffer, and stained with FITC-conjugated annexin-V and propidium iodide. The cells were stained according to the manufacturer's instructions and analyzed by flow cytometry. The histograms were analyzed with Kaluza Analysis Software v2.1 (Beckman Culture, Brea, CA, USA). The results are expressed as the percentage of apoptotic cells (annexin V^+ cells).

mRNA expression of pro-inflammatory cytokines and metaloproteinases after culture with sodium fluoride

The HFLS were seeded at 25 x 10^3 cells per well in 12-wells plate with 750 µL SGM and cultured until they reached 80% confluence. Then cells were starved in 750 µL serum-reduced medium (0,5% FBS in SBM) for 24 hours. After starvation medium were changed on 750 µL serum-reduced medium with (1 µM, 2,5 µM, 5 µM, 10 µM) or without sodium fluoride (control) for 6, 12 and 24 hours. Finally, the cells were washed with PBS and lysed with RLT buffer.

Total RNA was isolated using the Rneasy Mini Kit (QIAGEN, Valencia, CA, USA) and reverse-transcribed into cDNA using the First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) on a T100 thermal cycler (BIO-RAD, Philadelphia, PA, USA). The quantitative assessment of mRNA levels was performed by the real-time PCR on an ABI 7500 Fast instrument (Applied Biosystems, Foster City, CA, USA) with Power SYBR Green PCR Master Mix reagent (Applied Biosystems, Foster City, CA, USA). The primer sequences used for real time PCR analysis are shown in Table 1. The thermal cycling conditions were as follows: (1) 95°C for 15 seconds, (2) 40 cycles of 95°C for 15 seconds, (3) 60°C for 1 min. According to the melting point analysis, only one PCR product was amplified under those conditions. The relative expression of target genes was determined by the ΔCt method with β2-microglobulin as the internal reference control gene.

Statistical analysis

The statistical analysis was performed by Statistica 13.0 (StatSoft, Cracow, Poland). Results are expressed as mean ± standard error of mean (SEM) (from three independent experiments). The normality of variables was tested by the Shapiro-Wilk test. Statistical comparisons were performed using the Mann-Whitney U test. $p<0.05$ was considered statistically significant.

RESULTS

Proliferation

Figure 1 shows the absorbance values of the WST-1 test of HFLS after 24, 48 and 72 hrs incubation with (1-10 µM) or without (control) NaF. There were no significant differences in proliferative activities between HFLS cultured with NaF and the control culture ($p>0.05$). The results indicate that fluoride in the range of concentrations 1-10 µM has neither hyperproliferative nor antiproliferative effects in relation to HFLS.

Apoptosis

Cell apoptosis was evaluated by flow cytometry with Annexin-V/FITC staining, as shown in Figure 2. The cytometric analysis of cells was performed after 24, 48 and 72 hrs incubation with (1-10 µM) or without (control) NaF. There were no significant differences the in percentage of apoptotic cells between HFLS cultured with NaF and the control culture ($p>0.05$). This shows that fluoride in the range of concentrations 1-10 µM neither induces nor reduces apoptosis of HFLS cell.

mRNA expression of pro-inflammatory cytokines and metaloproteinases

We showed the significant increases in expression levels of TNF-α, IL-6, IL-8, MMP-3 and MMP-9 in response to NaF. The expression levels were dependent on time of exposure to NaF. The first significant increases in expression levels of all compounds were observed after 6 hrs incubation with 5 µM and 10 µM NaF.

The highest peak of TNF-α expression occurred after 12 hrs of incubation, when the significant increase in expression was for 2.5 µM ($p = 0.005$) and 5 µM ($p = 0.005$), but not for 10 µM ($p = 0.298$). These alterations were not observed after 24 hrs. Expression of IL-6 peaked after 24 hrs (5 µM, $p = 0.03$), but it should be noted that the effect of fluoride was most pronounced after 6 hrs (~4-, 5.5-, and 4.5-fold of control expression after 6 hrs with 2.5µM, 5 µM and 10 µM, respectively vs. ~2-fold of control expression after 24 hrs with 5 µM). After 12 and 24 hrs, the increase in IL-6 expression was only observed for 5 µM NaF ($p = 0.045$ for 12 h, $p = 0.03$ for 24 h).

IL-8 expression peaked after 6 hrs of incubation with 5 µM ($p = 0.01$) and 10 µM ($p = 0.008$)

NaF. The significant effect of these concentrations was maintained to 24 hrs (5 µM: p = 0.008 and 0.01, respectively for 12 and 24 hrs; 10 µM: p = 0.01 and 0.03, respectively for 12 and 24 hrs).

The peak MMP-3 expression appeared after 6 hrs with 10 µM (p = 0.02) NaF and after 12 hrs for 1 µM (p = 0.005), 2.5 µM (p = 0.008) and 5 µM (p = 0.005) NaF. The expression then decreased maintaining a significant increase only for 10 µM (p = 0.01). Similar dynamics of alterations in the expression was observed for MMP-9, with the difference that the significant increases in the expression after 24 hrs was maintained for 2.5 µM (p = 0.03), 5 µM (p = 0.02) and 10 µM (p = 0.045) NaF. Moreover, MMPs expressions was most strongly induced at the lowest concentration, but this effect was later and short-term (only after 12 hrs) compared to the higher NaF concentrations (p = 0.005 for both MMPs).

Table 1. Sequences of primers used for quantitative real time PCR analysis

Gene	Forward	Reverse
B2M	5'-AATGCGGCATCTTCAAACCT-3'	5'-TGACTTTGTCACAGCCCAAGATA-3'
TNF-α	5'-CCTCTCTCTAATCAGCCCTCTG-3'	5'-GAGGACCTGGGAGTAGATGAG-3'
IL-6	5'-AATTCGGTACATCCTCGACGG-3'	5'-GGTTGTTTTCTGCCAGTGCC-3'
IL-8	5'-ACTGAGAGTGATTGAGAGTGGAC-3'	5'-AACCCTCTGCACCCAGTTTTC-3'
MMP-3	5'-AGTCTTCCAATCCTACTGTTGCT-3'	5'-TCCCCGTCACCTCCAATCC-3'
MMP-9	5'-GAGATGTGCGTCTTCCCCTT-3'	5'-AGAATGATCTAAGCCCAGCGC-3'

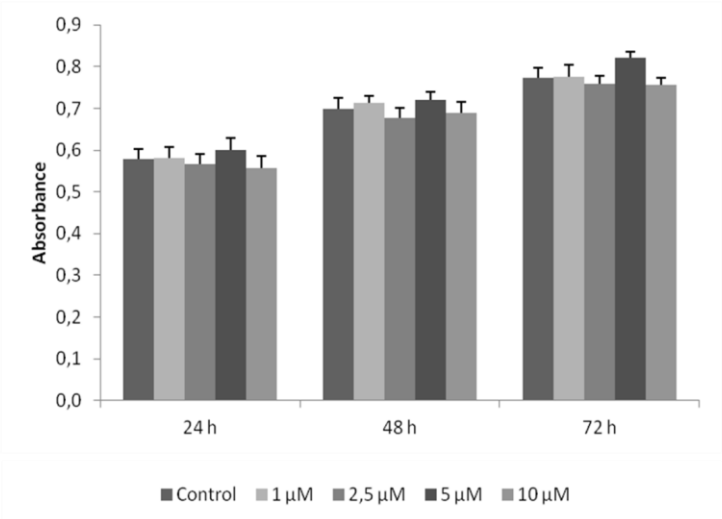

Figure 1. The effect of sodium fluoride on the proliferation of HFLS. HFLS were cultured with (1-10 µM) or without (control) NaF for 24, 48 and 72 hrs. The absorbance values of WST-1 assay were given as mean ± SEM. *p<0.05 compared to the control cell culture (Mann-Whitney *U* test).

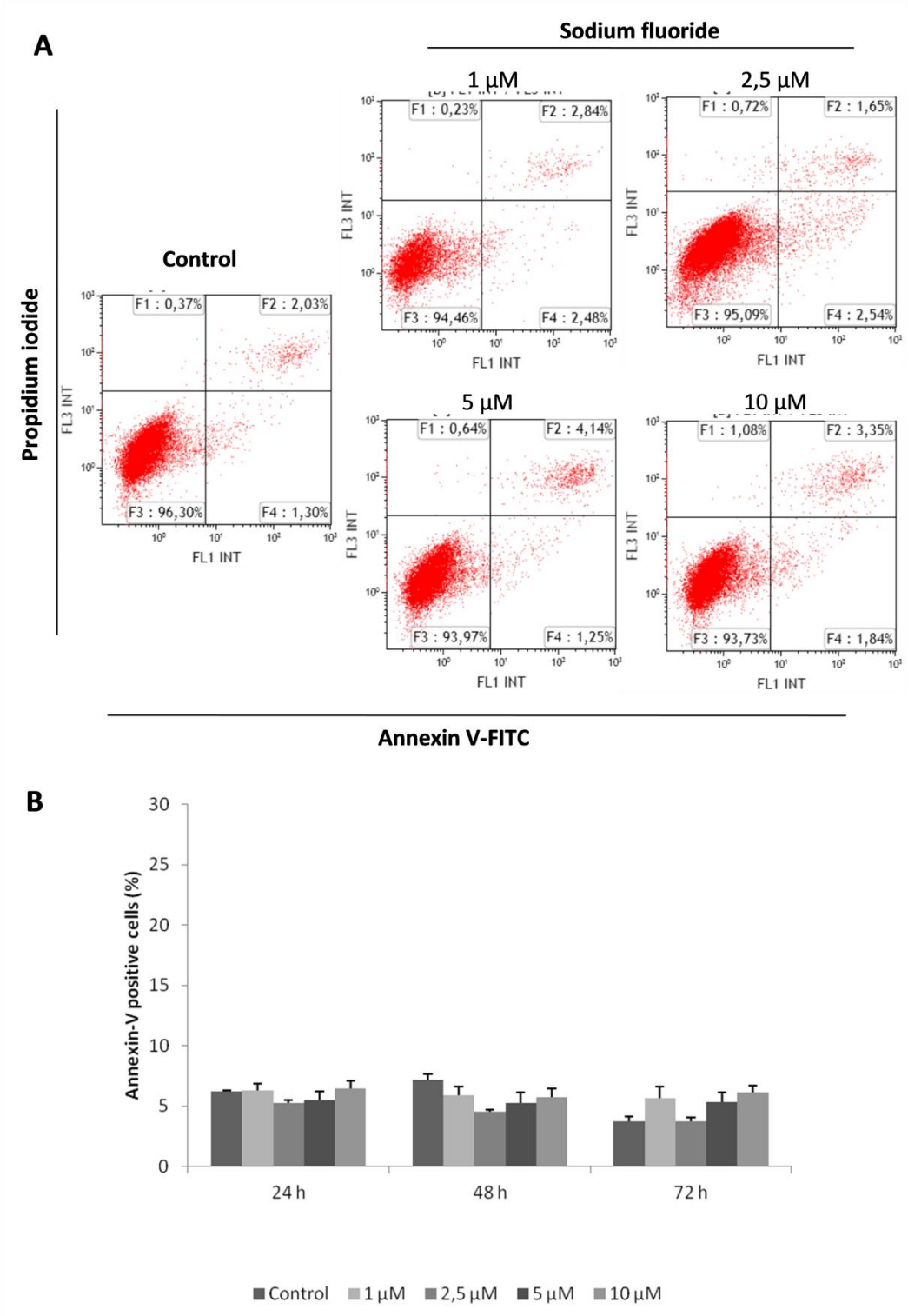

Figure 2. The effect of sodium fluoride on apoptosis of HFLS. HFLS were cultured with (1-10 µM) or without (control) sodium fluoride for 24, 48 and 72 hrs. The percentage of annexin-V positive cells were given as mean ± SEM. *p<0.05 compared to the control cell culture (Mann-Whitney *U* test).

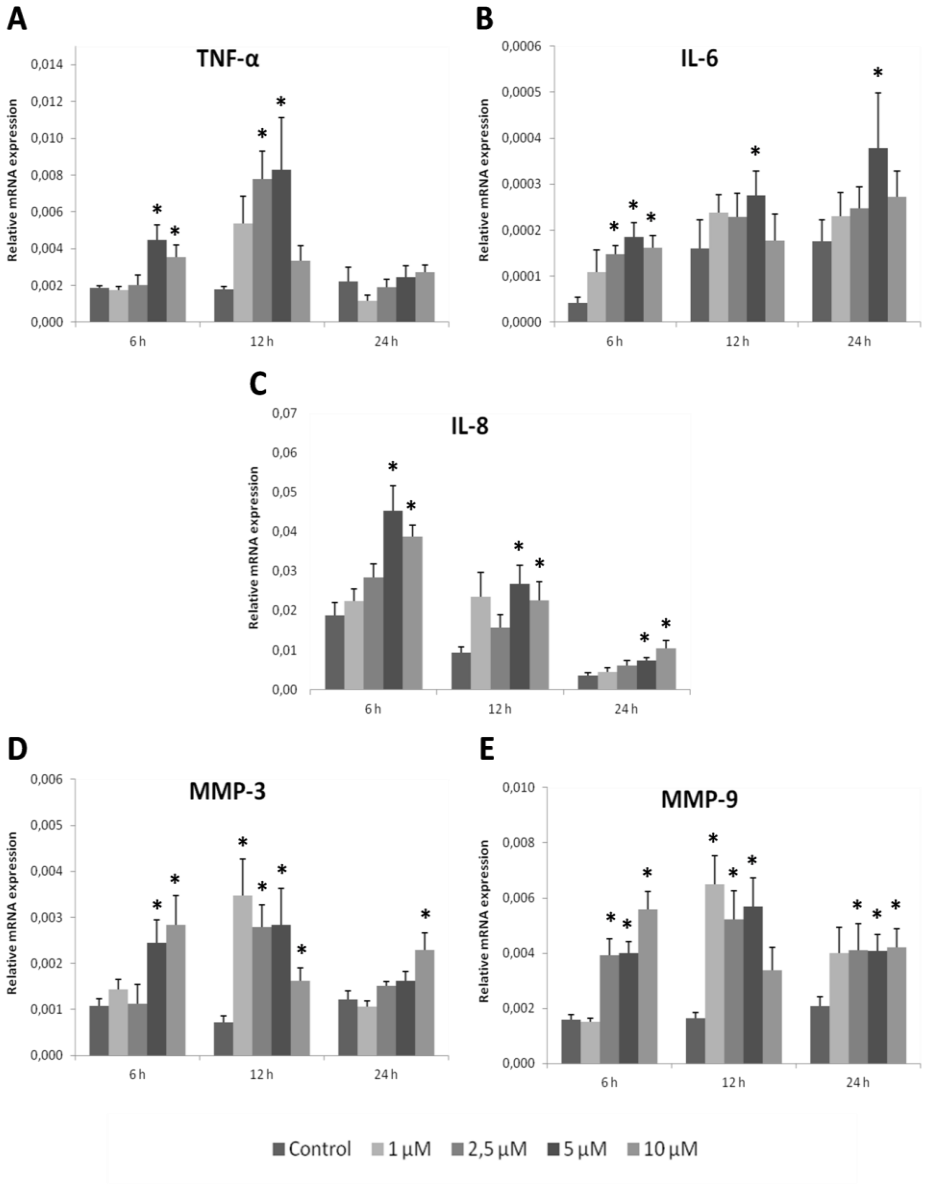

Figure 3. The effect of sodium fluoride on the mRNA expression of (A) TNF-α, (B) IL-6, (C) IL-8, (D) MMP-3 and (E) MMP-9 in HFLS. HFLS were cultured with (1-10 µM) or without (control) NaF for 6, 12 and 24 hrs. The relative mRNA expressions were given as mean ± SEM. *p<0.05 compared to the control cell culture (Mann–Whitney *U* test).

DISCUSSION

Excessive exposition of fluoride is a risk factor of arthritis.[7,9,14] We hypothesized that fluoride affects FLSs, one of the major populations of synovium cells. The pathological process involving the synovium is an important component of the pathogenesis of RA.[19] We found it interesting to investigate whether the same is true for joints disease associated with fluoride intoxication. Previously we reported that NaF (3-10 µM) triggered inflammatory response of macrophages.[20-23] We chose concentrations of fluoride ranging from the blood plasma levels of individuals from non-endemic regions (0.02-0.08 mg/L, 0.5-2 µM) to higher concentrations observed in individuals environmentally exposed to fluoride (>0.2 mg/L, >4.8 µM), as previously.[20-23]

This is the first research to evaluate the effects of fluoride on HFLS. In a normal state, FLSs play a key role in maintaining the homeostasis of joints – form normally organized synovial lining and release components of synovial fluid. In RA, phenotype of FLSs changes to tumor-like phenotype characterized by high proliferation, reduced apoptosis, increased expression of proinflammatory cytokines, chemokines, adhesions

molecules and MMPs. Joint microenvironment modified by FLSs promote recruitment of immune cells, inflammation and destruction of joint tissues.[25]

We first verified whether fluoride induced changes in FLS growth typical of arthritis. We found no confirmation that fluoride at concentration of 1-10 µM stimulated hyperproliferation or affected apoptosis of HFLS. Researchers have shown that the effect on cell growth is concentration-, time- and cell-dependent. The increase in proliferation was observed over a wide range of fluoride micromolar concentrations. Based on research on various types of cells, it was established that the pro-proliferative effect appears after exposure to sodium fluoride in the concentration range 10-500 µM.[26-30] The lack of HFLS apoptosis induction is not surprising. The proapoptotic effect of fluoride is rather seen at millimolar concentrations in laboratory conditions and initiated through mitochondrion-, endoplasmic reticulum stress- or death receptor-mediated pathways.[31-35] We also did not observe a reduction of apoptosis. It is likely that the observation of the pro-proliferative and anti-apoptotic effects of 5-10 µM NaF on HFLS is only visible at the molecular level or requires chronic exposure to NaF. This explanation is supported by Mendoza-Schulz et al.[26], who found only a discrete induction of DNA synthesis, without a change in cell number, at a concentration of about 10 µM.

Synovial inflammation is important feature of arthritis mediated by numerous biologically active compounds such as pro-inflammatory cytokines and chemokines. TNF-α and IL-6 are central mediators with multidirectional function in the early phase of joint inflammation.[36,37] TNF-α is an inducer of many arthritogenic genes, including IL-1β, IL-6, IL-8, CXCL-10[37], and MMPs associated with cartilage destruction.[38] IL-6 induces VEGF expression promoting angiogenesis, and then migration of immune cells. Infiltrating leukocytes produce additional pro-inflammatory cytokines and intensify inflammation.[37] Moreover, TNF-α together with IL-6 stimulate osteoclastogenesis, which promotes bone erosion.[36,37] Both cytokines are considered pain mediators.[39] In our study, increase in TNF-α and IL-6 expression were observed already after 6 h incubation with NaF and they were most pronounced at a concentration of 5 µM. The action of 10 µM NaF was shorter and weaker. An interesting observation was the increase in IL-8 expression after exposure to 5-10 µM NaF. IL-8 is another important player in arthritis due to its strong chemotactic activity on many different cells, including neutrophils and T cells.[37,40,41] Moreover, IL-8 promotes the hypertrophy of chondrocytes, the calcification of matrix, and increases the levels of MMP-1 and MMP-13.[42] The studies in animal models[43-46] and cell models[47-49] of fluoride intoxication support that fluoride induces an inflammatory response with an increase in the production of pro-inflammatory cytokines, including TNF-α, IL-6 and IL-8, and reactive oxygen species. However, it should be borne in mind that fluoride doses used in these researches were high and the researches concerned a different type of cells than in our study.

Another important component of arthritis is the destruction of articular cartilage mediated by MMPs derived from FLSs.[38] We showed the increases in the expression levels of MMP-3 and MMP-9 in HFLS after incubation with 5-10 µM NaF. So far, the increase in MMP-9 expression after exposure to NaF has been observed in osteoclasts in goldfish[50], and in the bones[51] and the central nervous system[52,53] in studies on rodent models. However, in the study published by Wad-dington and Langley, significant amounts of MMP-3 were demonstrated in preparations from rat bone cells cultured in the presence of fluoride (0.1 and 10 µM), whereas only trace amounts were detected in cultures incubated without fluoride.[54] We can suspect that the chronically sustained increase in the expression of these MMPs will lead to progressive joint destruction, such as in inflammatory diseases of the joints.[55] MMP-3 has broad range of substrates, including proteoglycans, fibronectin, laminin, collagen telopeptides and basal lamina collagen IV. Moreover, MMP-3 is a key procollagenase activator, which can bind to collagen and regulate its turnover.[56] MMP-9 participates in the final degradation stage leading to the loss of proteoglycans and collagen from cartilage.[57] Besides the induction of the MMPs expression by 5-10 µM concentrations, there was surprisingly a strong but short-term increase at the lowest NaF concentration. It is possible that this short-term effect of 1 µM NaF, unlike higher concentrations, will have beneficial consequences due to the participation of MMP-3 and MMP-9 in the formation and normal remodeling of bones and cartilages.[58] Benefits of treatment with low doses (≤20 mg/day of fluoride equivalents) of fluoride was described in osteoporotic patients. They include an increase in bone mineral density and a reduction of fracture risk associated with stimulation of bone formation.[59] It would be wise to look at whether low doses of fluoride may be of benefit in joint diseases.

CONCLUSIONS

In summary, our study supports the hypothesis that fluoride at 5-10 µM concentration induces the alterations of pro-inflammatory cytokines and MMPs expression in HFLS, which may favor creating an inflammatory milieu in the synovium and predisposing to joint damage.

FUNDING

Not applicable

CONFLICT OF INTERESTS

None

REFERENCES

[1] Kurdi MS. Chronic fluorosis: The disease and its anaesthetic implications. Indian J Anaesth 2016;60(3):157-62.doi:10.4103/0019-5049.177867.

[2] Saha A, Mukherjee AK, Ravichandran B. Musculoskeletal problems and fluoride exposure: A cross-sectional study among metal smelting workers. Toxicol Ind Health 2016;32(9):1581-8. doi:10.1177/0748233714568477.

[3] Gupta R, Kumar AN, Bandhu S, Gupta S. Skeletal fluorosis mimicking seronegative arthritis. Scand J Rheumatol 2007;36(2):154-5.

[4] Kumar S, Kakar A, Gogia A, Byotra SP. Skeletal fluorosis mimicking seronegative spondyloarthropathy: a deceptive presentation.Trop Doct 2011;41(4):247-8. doi: 10.1258/td.2011.110117.

[5] Shukla A. Skeletal fluorosis mimicking seronegative arthritis. Indian J Rheumatol 2016;11:171-3.

[6] Ugran V, Desai NN, Chakraborti D, Masali KA, Mantur P, Kulkarni S, et al. Groundwater fluoride contamination and ist possible health implications in Indi taluk of Vijayapura District (Karnataka State), India. Environ Geochem Health 2017;39:1017-29. doi.org/10.1007/s10653-016-9869-2.

[7] Death C, Coulson G, Kierdorf U, Kierdorf H, Ploeg R, Firestone S, et al. Chronic excess fluoride uptake contributes to degenerative joint disease (DJD): Evidence from six marsupial species. Ecotoxicol Environ Saf 2018;30(162):383-90.

doi: 10.1016/j.ecoenv.2018.06.062.

[8] Kosik-Bogacka D, Łanocha-Arendarczyk N, Kot K, Ziętek P, Karaczun M, Gutowska I, et al. Fluoride concentration in synovial fluid, bone marrow, and cartilage in patients with osteoarthritis. Fluoride 2018;51(2):164-70.

[9] Singh VK, Rathore KS, Khan G, Rahim A, Rashid A, Chauhan S. Clinical and Radiological Study of Serum Fluoride in Relation to Knee Osteoarthritis. Malays Orthop J 2020;14(3):151-4. doi: 10.5704/MOJ.2011.023.

[10] Savas S, Cetin M, Akdoğan M, Heybeli N. Endemic fluorosis in Turkish patients: relationship with knee osteoarthritis. Rheumatol Int 2001;21(1):30-5.

[11] Qin X, Wang S, Yu M, Zhang L, Li X, Zuo Z, et al. Child skeletal fluorosis from indoor burning of coal in southwestern China. J Environ Public Health 2009;969764. doi: 10.1155/2009/ 969764.

[12] Ramesh M, Malathi N, Ramesh K, Aruna RM, Kuruvilla S. Comparative Evaluation of Dental and Skeletal Fluorosis in an Endemic Fluorosed District, Salem, Tamil Nadu. J Pharm Bioallied Sci 2017;9 Suppl 1:S88-S91. doi:10.4103/jpbs.JPBS_77_17.

[13] Mohammadi AA, Yousefi M, Yaseri M, Jalilzadeh M, Mahvi AH. Skeletal fluorosis in relation to drinking water in rural areas of West Azerbaijan, Iran. Sci Rep 2017;11;7(1):17300.

doi: 10.1038/s41598-017-17328-8.

[14] Wang J, Li H, Pei J, Gao Y. Association Between Osteoarthritis and Water Fluoride Among Tongyu Residents, China, 2019: a Case-Control of Population-Based Study. Biol Trace Elem Res 2022;200(7):3107-16. doi: 10.1007/s12011-021-02937-2.

[15] Melaku Z, Assefa G, Enqusilassie F, Bjorvatn K, Tekle-Haimanot R. Epidemiology of skeletal fluorosis in Wonji Shoa Sugar Estate, Wonji, Ethiopia: a community based survey. Ethiop Med J 2012;50(4):307-13.

[16] Jarvis HG, Heslop P, Kisima J, Gray WK, Ndossi G, Maguire A, et al. Prevalence and aetiology of juvenile skeletal fluorosis in the south-west of the Hai district, Tanzania community-based prevalence and case-control study. Trop Med Int Health 2013;18(2):222-9. doi:10.1111/tmi.12027.

[17] Wei W, Pang S, Sun D. The pathogenesis of endemic fluorosis: Research progress in the last 5 years. J Cell Mol Med 2019;19.

doi: 10.1111/jcmm.14185.

[18] Rezaee T, Bouxsein ML, Karim L. Increasing fluoride content deteriorates rat bone mechanical properties. Bone 2020;136: 115369. doi: 10.1016/j.bone.2020.115369.

[19] Tu J, Hong W, Zhang P, Wang X, Körner H, Wei W. Ontology and Function of Fibroblast-Like and Macrophage-Like Synoviocytes: How Do They Talk to Each Other and Can They Be Targeted for Rheumatoid Arthritis Therapy? Front Immunol 2018;26;9:1467.

doi: 10.3389/fimmu.2018.01467.

[20] Gutowska I, Baranowska-Bosiacka I, Baśkiewicz M, Millo B, Siennicka A, Marchlewicz M, et al. Fluoride as a pro-inflammatory factor and inhibitor of ATP bioavailability in differentiated human THP1 monocytic cells. Toxicol Lett 2010;1;196(2):74-9.

doi: 10.1016/j.toxlet.2010.03.1167.

[21] Gutowska I, Baranowska-Bosiacka I, Goschorska M, Kolasa A, Łukomska A, Jakubczyk K, et al. Fluoride as a factor initiating and potentiating inflammation in THP1 differentiated monocytes/ macrophages. Toxicol In Vitro 2015;29(7):1661-8.

doi: 10.1016/j.tiv.2015.06.024.

[22] Gutowska I, Baranowska-Bosiacka I, Safranow K, Jakubowska K, Olszewska M, Telesiński A, et al. Fluoride in low concentration modifies expression and activity of 15 lipoxygenase in human PBMC differentiated monocyte/macrophage. Toxicology 2012;16;295(1-3):23-30. doi: 10.1016/j.tox.2012.02.014.

[23] Gutowska I, Baranowska-Bosiacka I, Siennicka A, Baśkiewicz M, Machaliński B, Stachowska E, et al. Fluoride and generation of pro-inflammatory factors in human macrophages. Fluoride 2011;44(3):125-34.

[24] Bustamante MF, Garcia-Carbonell R, Whisenant KD, Guma M. Fibroblast-like synoviocyte metabolism in the pathogenesis of rheumatoid arthritis. Arthritis Res Ther 2017;19(1):110.

doi: 10.1186/s13075-017-1303-3.

[25] Mousavi MJ, Karami J, Aslani S, Tahmasebi MN, Vaziri AS, Jamshidi A, et al. Transformation of fibroblast-like synoviocytes in rheumatoid arthritis; from a friend to foe. Auto Immun Highlights 2021;12(1):3. doi: 10.1186/ s13317-020-00145-x.

[26] Mendoza-Schulz A, Solano-Agama C, Arreola-Mendoza L, Reyes-Márquez B, Barbier O, Del Razo LM, et al. The effects of fluoride on cell migration, cell proliferation, and cell metabolism in GH4C1 pituitary tumour cells. Toxicol Lett 2009;190(2):179-86. doi: 10.1016/j.toxlet.2009.07.014.

[27] He H, Wang H, Jiao Y, Ma C, Zhang H, Zhou Z. Effect of Sodium Fluoride on the Proliferation and Gene Differential Expression in Human RPMI8226 Cells. Biol Trace Elem Res 2015;167:11-7.

[28] Li KQ, Jia SS, Ma M, Shen HZ, Xu L, Liu GP, et al. Effects of fluoride on proliferation and mineralization in periodontal ligament cells in vitro. Braz J Med Biol Res 2016;11;49(8):e5291. doi: 10.1590/1414-431X20165291.

[29] Gu X, Wang Z, Gao J, Han D, Zhang L, Chen P, et al. SIRT1 suppresses p53-dependent apoptosis by modulation of p21 in osteoblast-like MC3T3-E1 cells exposed to fluoride. Toxicol In Vitro 2019;57:28-38. doi: 10.1016/j.tiv.2019.02.006.

[30] Bhawal UK, Li X, Suzuki M, Taguchi C, Oka S, Arikawa K, et al. Treatment with low-level sodium fluoride on wound healing and the osteogenic differentiation of bone marrow mesenchymal stem cells. Dent Traumatol 2020;36(3):278-84. doi: 10.1111/ edt.12532.

[31] Jeng J, Hsieh C, Lan W, Chang M, Lin SK, Hahn LJ, et al. Cytotoxicity of sodium fluoride on human oral mucosal fibroblasts and its mechanisms. Cell Biol Toxicol 1998;14,383-9. doi.org/10.1023/ A:1007591426267.

[32] Agalakova NI, Gusev GP. Molecular Mechanisms of Cytotoxicity and Apoptosis Induced by Inorganic Fluoride. Int Schol Res Notices 2012;403835. doi.org/10.5402/2012/403835.

[33] Wei Q, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. A mini review of fluoride-induced apoptotic pathways. Environ Sci Pollut Res 2018;25,33926-35. doi.org/10.1007/s11356-018-3406-z

[34] Johnston NR, Strobel SA. Principles of fluoride toxicity and the cellular response: a review. Arch Toxicol 2020;94(4):1051-69. doi: 10.1007/s00204-020-02687-5.

[35] Yu FF, Zuo J, Fu X, Gao MH, Sun L, Yu SY, et al. Role of the hippo signaling pathway in the extracellular matrix degradation of chondrocytes induced by fluoride exposure. Ecotoxicol Environ Saf 2021;225:112796. doi: 10.1016/j.ecoenv.2021.112796.

[36] Lin YJ, Anzaghe M, Schülke S. Update on the Pathomechanism, Diagnosis, and Treatment Options for Rheumatoid Arthritis. Cells 2020;9(4):880. doi: 10.3390/cells9040880.

[37] Kondo N, Kuroda T, Kobayashi D. Cytokine Networks in the Pathogenesis of Rheumatoid Arthritis. Int J Mol Sci 2021;22(20): 10922. doi: 10.3390/ijms222010922.

[38] Tateiwa D, Yoshikawa H, Kaito T. Cartilage and Bone Destruction in Arthritis: Pathogenesis and Treatment Strategy: A Literature Review. Cells 2019;8(8):818. doi: 10.3390/cells8080818.

[39] Schaible HG. Nociceptive neurons detect cytokines in arthritis. Arthritis Res Ther 2014;16(5):470. doi: 10.1186/s13075-014- 0470-8.

[40] Russo RC, Garcia CC, Teixeira MM, Amaral FA. The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. Expert Rev Clin Immunol 2014;10(5):593-619. doi: 10.1586/1744666X.2014.894886.

[41] Molnar V, Matišić V, Kodvanj I, Bjelica R, Jeleč Ž, Hudetz D, et al. Cytokines and Chemokines Involved in Osteoarthritis Pathogenesis. Int J Mol Sci 2021;22(17):9208. doi: 10.3390/ ijms22179208.

[42] Merz D, Liu R, Johnson K, Terkeltaub R. IL-8/CXCL8 and growth-related oncogene alpha/CXCL1 induce chondrocyte hypertrophic differentiation. J Immunol 2003;171(8):4406-15. doi: 10.4049/jimmunol.171.8.4406.

[43] Ma Y, Niu R, Sun Z, Wang J, Luo G, Zhang J, et al. Inflammatory responses induced by fluoride and arsenic at toxic concentration in rabbit aorta. Arch Toxicol 2012;86(6):849-56. doi: 10.1007/ s00204-012-0803-9.

[44] Wei R, Luo G, Sun Z, Wang S, Wang J. Chronic fluoride exposure-induced testicular toxicity is associated with inflammatory response in mice. Chemosphere 2016;153:419-25. doi: 10.1016/j.chemosphere.2016.03.045.

[45] Luo Q, Cui H, Deng H, Kuang P, Liu H, Lu Y, et al. Sodium fluoride induces renal inflammatory responses by activating NF-κB signaling pathway and reducing anti-inflammatory cytokine expression in mice. Oncotarget 2017;8(46):80192-207. doi: 10.18632/oncotarget.

[46] Chen L, Kuang P, Liu H, Wei Q, Cui H, Fang J, et al. Sodium Fluoride (NaF) Induces Inflammatory Responses Via Activating MAPKs/NF-κB Signaling Pathway and Reducing Anti-inflammatory Cytokine Expression in the Mouse Liver. Biol Trace Elem Res 2019;189(1):157-71. doi: 10.1007/s12011-018-1458-z.

[47] Yan L, Liu S, Wang C, Wang F, Song Y, Yan N, et al. JNK and NADPH oxidase involved in fluoride-induced oxidative stress in BV-2 microglia cells. Mediators Inflamm 2013;2013:895975. doi: 10.1155/2013/895975.

[48] Refsnes M, Skuland T, Låg M, Schwarze PE, Øvrevik J. Differential NF-κB and MAPK activation underlies fluoride- and TPA-mediated CXCL8 (IL-8) induction in lung epithelial cells. J Inflamm Res 2014;7:169-85. doi: 10.2147/JIR.S69646.

[49] Zhang Y, Zhou BH, Tan PP, Chen Y, Miao CY, Wang HW. Key Role of Pro-inflammatory Cytokines in the Toxic Effect of Fluoride on Hepa1-6 Cells. Biol Trace Elem Res 2020;197(1):115-22. doi: 10.1007/s12011-019-01967-1.

[50] Sato M, Hanmoto T, Yachiguchi K, Tabuchi Y, Kondo T, Endo M, et al. Sodium fluoride induces hypercalcemia resulting from the upregulation of both osteoblastic and osteoclastic activities in goldfish, Carassius auratus. Comp Biochem Physiol C Toxicol Pharmacol 2016;189:54-60. doi: 10.1016/j.cbpc.2016.07.006.

[51] Wei Y, Zeng B, Zhang H, Chen C, Wu Y, Wang N, et al. Comparative proteomic analysis of fluoride treated rat bone provides new insights into the molecular mechanisms of fluoride toxicity. Toxicol Lett 2018;291:39-50. doi: 10.1016/j.toxlet.2018.04.006.

[52] Qing-Feng S, Ying-Peng X, Tian-Tong X. Matrix metalloproteinase-9 and p53 involved in chronic fluorosis induced blood-brain barrier damage and neurocyte changes. Arch Med Sci 2019;15(2):457-66. doi: 10.5114/aoms.2019. 83294.

[53] Łukomska A, Baranowska-Bosiacka I, Dec K, Pilutin A, Tarnowski M, Jakubczyk K, et al. Changes in Gene and Protein Expression of Metalloproteinase-2 and -9 and Their Inhibitors TIMP2 and TIMP3 in Different Parts of Fluoride-Exposed Rat Brain. Int J Mol Sci 2020;22(1):391. doi: 10.3390/ijms22010391.

[54] Waddington RJ, Langley MS. Altered expression of matrix matalloproteinases within mineralizing bone cells in vitro in the presence of fluoride. Connect Tissue Res 2003;44(2):88-95.

[55] Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. Int J Mol Sci 2020;21(24):9739. doi: 10.3390/ ijms21249739.

[56] Manka SW, Bihan D, Farndale RW. Structural studies of the MMP-3 interaction with triple-helical collagen introduce new roles for the enzyme in tissue remodelling. Sci Rep 2019;9(1):18785. doi: 10.1038/s41598-019-55266-9.

[57] Malemud CJ. Matrix Metalloproteinases and Synovial Joint Pathology. Prog Mol Biol Transl Sci 2017;148:305-25. doi: 10.1016/bs.pmbts.2017.03.003.

[58] Löffek S, Schilling O, Franzke CW. Series "matrix metalloproteinases in lung health and disease": Biological role of matrix metalloproteinases: a critical balance. Eur Respir J 2011;38(1):191-208. doi: 10.1183/09031936.00146510.

[59] Vestergaard P, Jorgensen NR, Schwarz P, Mosekilde L. Effects of treatment with fluoride on bone mineral density and fracture risk--a meta-analysis. Osteoporos Int 2008;19(3):257-68. doi: 10.1007/s00198-007-0437-6.