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Research Article

Effect of Some Vitamins on Antioxidant/Prooxidant Parameters in Sodium Fluoride (NaF)-Treated Cell Line (hFOB 1.19)

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ABSTRACT

Objective: This study was planned to determine the effect of certain vitamin applications on antioxidant and oxidant parameters in the osteoblast cell line exposed to sodium fluoride *in vitro* and to evaluate the protective role of certain vitamins against possible toxic effects of fluoride.
Materials and Methods: Cells were replicated *in vitro* conditions with regular passaging 2-3 times weekly. MTF viability test was used to determine IC_{50} of NaF (5000µM) and proliferative doses of vitamins (Vitamin A: 10µM, Vitamin D: 10µM, Vitamin E: 60µM, Vitamin C: 100µM) for hFOB 1.19 cells. Cells were sown in flasks as so to be 10^6. The study groups were identified as control, NaF, vitamins and NaF+vitamins. After incubation for 24 hours, cells treated with trypsin were prepared by freeze/thaw method and MTT viability test, TAS, SOD, GSH, CAT, TOS and MDA analyzes were performed on these samples.
Results: In the hFOB 1.19 cell line, TAS levels decreased significantly in the NaF group ($p \leq 0.05$), but were close to the control group in NaF+vitamin groups with the exception of vitamin C. However, there was no difference between the groups in terms of GSH level and CAT and SOD activities when the control and NaF groups were compared. It was observed that TOS level increased significantly in the NaF group ($p<0.05$), decreased in the NaF+vitamin groups and were lower in the NaF+vitamin C and E groups than the control group ($p <0.05$). While OSI was the highest in the NaF group, no significant difference in MDA level was observed compared with the control group.
Conclusion: As a result, it was found that NaF administration in the osteoblast cell line increased oxidative stress and decreased following vitamin application. It was found that the effect of NaF administration in the osteoblast cell line on cell viability was consistent with the oxidative stability and that the vitamin application conformably changed cell viability and oxidative balance.

Keywords: NaF, cell culture, antioxidant, oxidative state, vitamins.

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INTRODUCTION

Fluoride (F) is an element with high electronegativity that can be found naturally in water and in various nutrients. Long-term, high-concentration exposure causes damage to teeth, bones, and various tissues (Perumal et al., 2013). The surplus of flour is confronted as an important problem threatening human and animal health in many parts of the world and in our country (in the neighborhood of Tendürek and Isparta etc.) (Altıntaş et al., 2000; Yur et al., 2013).

Living cells must have many defense mechanisms to fight with ROS (reactive oxygen species) and other free radicals. The simplest of these defense mechanisms are antioxidants such as vitamin C and vitamin D with low molecular weight, which interact with free radicals and make them less reactive to protect cellular biomolecules from damages. Vitamin A, C, and E help protect the organism from free radical oxidation in fluorosis. Vitamin D also plays a role in the recovery of fluoro-induced toxicity. Vitamin D has been revealed to show protective effect in cell and animal studies performed by many research groups (Chlubek and Poland, 2003; Cooke et al, 2006; Chaverri et al., 2016).

The measurement of total antioxidant capacity (TAS) is the sum of all antioxidant states. It is evaluated as the known and unknown antioxidant capacity and their synergistic effects (Ghisellia et al., 2000). Total oxidant capacity (TOS) is expressed as a whole of many oxidative stress products such as reactive nitrogen species (RNT), hydrochloric acid, malondialdehyde (MDA) and lipid peroxides in serum (Erel, 2005).

Many studies have been conducted on cellular and metabolic bases of flora-related toxicity (Lee et al., 2008; Yang et al., 2015; Zhang et al., 2007). Oxidative stress is also defined as a damage mechanism caused by fluoride exposure. Oxidative stress, emerging in response to flour exposure, has been reported to emerge in different cell types, tissues, and in humans and animals that live in endemically flourish areas (Gutiérrez-Salinas et al., 2013; Yur et al., 2013; Çetin et al., 2017; Yüksek et al., 2017).

This study was designed to determine the role of antioxidant/oxidant status in cell viability and the effects of some vitamin applications on fluoride-treated osteoblast cell line.

MATERIALS AND METHODS

Cell Material and Culture

The human bone hFOB 1.19 (ATCC® CRL-11372™) cell line was used in the study. hFOB 1.19 cells were propagated in a medium containing 37 °C, 5% CO_2 and 95% humidity and Ham's F12 feedlot containing 10% FBS and 1% penicillin/streptomycin 2 mM L-glutamine. hFOB 1.19 cells were passaged at a cell density of 70-80% confluency in the flask. Passaging was performed using Trypsin-EDTA (0.25%-0.1%).

Cell Viability Test (MTT)

IC_{50} (5000 μM) of NaF and the proliferative doses of vitamins (A: 10μM, D: 10μM, E: 60μM, C: 100μM) for hFOB 1.19 cell were determined by presuming the control group 100% viable by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide). In addition, for the effect of some vitamins (A: 10μM, D: 10μM, E: 60μM, C: 100μM) on various doses of NaF (100μM, 250μM, 500μM 1000μM, 2000μM, 5000μM, 7500μM, 10000μM), MTT viability test was performed according to the control group by using each dose of flour and each vitamin separately.

Study Groups

The cells were seeded in 96 culture plates as so to be 10^4 and in 25 cm^2 flasks as so to be 10^6. The study groups were determined as control, NaF, vitamin A, D, E, C, NaF+vitamin A, NaF+vitamin D, NaF+vitamin E and NaF+vitamin C. The doses were administered to the cells according to the designated groups.

Preparation of the Samples

After incubation for 24 hours, cells were collected by trypsinization and lysed by freeze/thaw method. The supernatant portion of the cell lysate was removed for analysis after centrifugation at 3000 rpm for 20 min.

Biochemical Analysis

TAS/TOS (Rel Assay: AT15053A-JY15060O) and SOD (Cayman) were determined in the obtained cell lysate by ELISA using a commercial kit. MDA (Ledwozyw et al., 1986) and GSH levels (Beutler et al., 1963) and CAT (Lartillot et al. 1988) activity were measured spectrophotometrically.

Statistical Analysis

The results were given as mean ± SD. SPSS (Version 20.00) was used for the statistical analysis of the data. Statistical analysis was assessed by ANOVA test. Values of $p<0.05$ were considered statistically significant.

RESULTS

The study showed that the highest increases in cell viability were observed in the vitamin A and D groups, together with the NaF concentrations selected as the study dose. Although the cell viability increased in

groups where 10 μM vitamin E was applied with NaF compared to the NaF groups, it was determined that cell viability of NaF+vitamin E groups decreased compared to NaF groups from 2000 μM dosing. In the groups treated with NaF and 100 μM vitamin C, cell viability generally decreased with respect to NaF groups, and the cell viability of NaF+vitamin C group increased compared to NaF group at 1000 μM dose.

TOS levels were found to increase significantly in the NaF group ($p \leq 0.05$), to decrease in all the experimental groups with NaF and vitamin and to be lower ($p \leq 0.05$) in the NaF+vitamin C and E groups than the control group. It was found that OSI is the highest in the NaF group, and was close to control group in the vitamin groups with the exception of NaF+vitamin C group.

Although the NaF group MDA level was higher than the control group, this difference was not significant. MDA levels were significantly lower than control group in both NaF group and normal osteoblast cells treated with vitamin C. The control group MDA level was found to be significantly higher than the groups treated with the vitamins alone as well as the NaF and vitamins.

Catalase Activity in Osteoblast Cells

There was no significant difference between the control group and the NaF-treated group with regard to the catalase activity. The catalase activity in the group treated with NaF and vitamin C was found to be significantly higher only in the control and NaF groups.

		Vitamin A	Vitamin D	Vitamin E	Vitamin C
Control	100	100,05	107,78	113,91	112,73
NaF (100μM)	90,00	86,46	98,01	100,59	84,73
NaF (250μM)	101,20	99,83	91,60	103,07	88,69
NaF (500μM)	95,82	116,15	95,19	93,63	83,63
NaF (1000μM)	83,21	87,6	100,12	91,71	91,98
NaF (2000μM)	75,40	80,17	77,73	60,68	63,30
NaF (5000μM)	53,67	56,26	50,78	41,07	43,46
NaF (7500μM)	35,26	36,22	37,11	25,85	30,78
NaF (10000μM)	9,98	22,52	26,61	11,26	10,67

Table 1: MTT results

TAS levels decreased significantly in the NaF group ($p \leq 0.05$). In the vitamins D and E groups, TAS level was significantly higher than control group ($p \leq 0.05$). In the NaF+vitamin groups, TAS levels were found to be closer to control group except for vitamin C.

GSH Level in Osteoblast Cells

In NaF alone group, GSH level was significantly lower than control group. While GSH level was significantly lower in vitamin alone group (Vitamin, A, Vitamin D, Vitamin C) compared to the NaF group, there was no statistically significant difference in the vitamin E treated group. The GSH level of the control group was significantly higher than vitamin E and vitamin C groups, but significantly lower than vitamin A and vitamin D groups. In groups treated with NaF and vitamins together, GSH level was found to be significantly lower in the groups treated with vitamin A than in the NaF group, but was found significantly higher in the group treated with vitamin C and vitamin A.

MDA Level in Osteoblast Cells

SOD Activity in Osteoblast Cells

There was no significant difference in SOD activity between osteoblast control and NaF group. While there was no significant difference in NaF+vitamin D group, SOD activity was significantly higher in the VaF+vitamin D group than in all other groups.

DISCUSSION

In the osteoblast cell line in which the flour was applied *in vitro*, the effects of applying both vitamin alone and with NaF as preservatives on cell viability were evaluated.

Reactive oxygen species interfere with the pathology of many diseases. Increases in reactive oxygen species and elevation of lipid peroxidation are thought to be responsible for toxicities of many compounds (Halliwelland and Gutteridge, 1986; Cross, 1987). Free radicals react with methyl groups of polyanionic fatty acids by generating malondialdehyde, one of the

	MDA	TOS	OSI	TAS	GSH	Catalase	SOD
Control	30.216±1.495 ^{e}	4.9281±1.1215^{d}	2.3711±0.4216^{c}	0.2064±0.0106^{b}	0.428±0.023 ^{e}	8.306±0.045 ^{a,b}	85.323±1.866 ^{c}
NaF	31.411±0.871 ^{e}	5.8633±0.7061^{e}	6.7999±0.2975^{e}	0.0860±0.0066^{a}	0.363±0.037^{c,d}	8.294±0.051 ^{a,b}	86.664±3.710 ^{c,d}
Vitamin A	17.372±1.712 ^{c}	4.3525±0.0415^{bcd}	2.3922±0.9211^{c}	0.2053±0.0808^{b}	0.584±0,029 ^{f}	8.225±0.068 ^{a}	80.995±1.375^{a,b}
Vitamin D	20.089±1.136 ^{d}	3.5971±0.0831^{ab}	1.0718±0.2171^{a}	0.3475±0.0781^{c}	0.568±0.031 ^{f}	8.217±0.070 ^{a}	81.856±1.927 ^{b}
Vitamin E	20.978±1.187 ^{d}	4.7122±0.4569^{cd}	1.5853±0.4098^{ab}	0.3188±0.1112^{c}	0.379±0.021 ^{d}	8.318±0.029 ^{a,b}	85.246±0.647 ^{c}
Vitamin C	15.260±0.624 ^{a,b}	3.1295±0.4569^{a}	1.9254±0.5241^{bc}	0.1669±0.0217^{b}	0.195±0.050 ^{a}	8.336±0.241 ^{a,b}	81.872±1.338 ^{b}
NaF +Vitamin A	19.778±0.899 ^{d}	4.4759±0.1015^{cd}	1.8503±0.0431^{bc}	0.2419±0.0066^{b}	0.294±0.020 ^{b}	8.312±0.032 ^{a,b}	82.451±0.575 ^{b}
NaF +Vitamin D	16.541±0.766 ^{b,c}	4.4245±0.4569^{cd}	2.2621±0.6235^{bc}	0.2029±0.0358^{b}	0.441±0.014 ^{e}	8.333±0.034 $^{a, b}$	88.996±1.456 ^{d}
NaF +Vitamin E	15.945±0.531 ^{a,b,c}	3.9208±0.1246^{bc}	2.2505±0.1057^{bc}	0.1743±0.0027^{b}	0.327±0.021 ^{b,c}	8.262±0.044 ^{a}	78.960±1.114 ^{a}
NaF +Vitamin C	14.496±0.490 ^{a}	3.9928±0.0415^{bc}	5.4646±0.4509^{d}	0.0734±0.0053^{a}	0.612±0.021 ^{f}	8.415±0.042 ^{b}	85.302±0.579 ^{c}

Table 2: TAS/TOS values, GSH and MDA levels, catalase and SOD activities in osteoblast

end products, which initiates peroxidation in the lipid membrane. Determining the level of MDA is a good measure of peroxidation, which is among the major cell damage mechanisms leading to necrosis and apoptosis (Singh, 1984; Mullenix et al., 1995). Many authors have investigated the relationship between fluoride and free radical reactions (Vani and Reddy, 2000; Zhi-Zhong et al., 1989; Kumari and Rao, 1991). It has been shown that flour causes increased lipid peroxidation *in vivo* and *in vitro* in tissue and blood of experimental animals and human erythrocytes (Zhi-Zhong et al., 1989; Sharma and Chinoy, 1998). Shanthakumari et al. (2004) showed that the levels of MDA were increased in the fluor-applied rat liver and kidney, and noted that the increase in lipid peroxidation may be due to increased oxidative stress as a result of the decrease of antioxidant systems. In some studies, while MDA levels induced by oxidative stress have been reported to increase in some tissues after low-dose fluoride application, it has been reported to decrease in some tissues at high doses (Chulebek, 2003; Soni et al., 1984). In the present study, although the level of MDA in the NaF group was higher than the control group, this difference was not significant. Following administration of Vitamin C, MDA levels in both NaF group and normal osteoblast cells were found to be significantly lower than control. Interestingly, the control group MDA level was determined to have significantly increased in all study groups. In the NaF-treated group, the TOS level was found to be significantly higher than that of the controls, consistent with the increase in MDA level.

Studies investigating the protective effect of vitamins against the oxidative stress caused by the application of Flour (F) have been carried out. In a study investigating the role of vitamins in the oxidative stress caused by the endometrial tissue in the flour endometrial tissue, it has been determined that endometrial MDA decreased; SOD, GSH-Px and CAT activities increased significantly; fluoride-induced endometrial damage decreased in both biochemical and histologic levels with the use of vitamin (Güney et al. 2007).

Vitamin E reinforcement has been shown to have a curative effect on histological and immunohistochemical changes in the frontal cortex neurons and glial tissue resulting from chronic fluorosis (Fattah et al., 2010).

Reduced glutathione (GSH) is known to protect cellular systems against the toxic effects of lipid peroxidation. GSH functions as a cofactor for many enzymes in capturing free radicals as co-substrate of GPx and as conjugate forms in endo and xenobiotic reactions (Nicoterave Orrrenius, 1986; Gregus et al., 1996). Increase in the lipid peroxidation and decrease in the level of GSH and so in the antioxidant system are reported to occur with flour application (Sies et al., 1993). Although the GSH level in the presented study was lower in the NaF group than the control, this difference was not significant. In the NaF-induced and

vitamin C-treated group, the GSH level was found to be significantly higher than the NaF group. Depending on the flour, GSH levels in this cell line are thought to depend on the dose and especially on the duration of application.

SOD is an important defense enzyme that converts superoxide radicals to hydrogen peroxides (Mc-Cord et al., 1984). Catalase compensates hydrogen peroxide and is protective against reactive hydroxyl radicals (Chance et al., 1982). It has been reported that SOD activities reduced in tissues exposed to high flour (McCord et al., 1984; Shanthakumari et al., 2004). Vani and Reddy (2000) reported that SOD and CAT activities decreased in brain and gastrocnemius muscle of NaF-treated rats. Catalase and SOD activities in the presented study did not show a significant difference when the NaF group and the control group were compared. However, it was noted that the SOD activity in the NaF+vitamin A group was the lowest. Although the SOD and CAT enzymes, of the parameters of antioxidant system, were not significantly affected by NaF alone application, the level of TAS was found to be significantly lower in the NaF group than the control group. It is thought that this situation has emerged with the influence of other parameters forming TAS. In the NaF+vitamin groups, the TAS level was shown to be no different from the control group. SOD activity was found to be significantly lower in NaF+vitamin E-treated group compared to control and NaF-treated group. SOD activity was found to be significantly higher in both NaF+vitamin D-treated group compared to the NaF and control groups, but significantly lower in vitamin D alone group. SOD and CAT activities were not significantly different from control and NaF groups when the vitamins were administered alone or in combination with NaF.

As a result, it was determined that NaF administration in the osteoblastic cell line increased oxidative stress and decreased after vitamin application. In reaching this result, rather than the MDA, SOD CAT and GSH parameters, TAS and TOS were found to be more determinative; and in flora-related oxidative damage, the role of more different oxidative and antioxidant parameters were was found to be more significant. It was also concluded that the cell viability in the experimental groups was compatible with the oxidative balance and that the change of oxidative balance in cell death and viability due to NaF application was effective.

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