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Impact of fluoride and a static magnetic field on the gene expression that is associated with the antioxidant defense system of human fibroblasts

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ABSTRACT

Fluoride cytotoxicity has been associated with apoptosis, oxidative stress, general changes in DNA and RNA and protein biosynthesis, whereas the results of studies on the effect of SMF on antioxidant activity of cells are contradictory. Therefore, the aim of our study was to evaluate the simultaneous exposure of human cells to fluoride SMF that are generated by permanent magnets on the expression profile of the genes that are associated with the antioxidant defense system. Control fibroblasts and fibroblasts that had been treated with fluoride were subjected to the influence of SMF with a moderate induction. In order to achieve our aims, we applied modern molecular biology techniques such as the oligonucleotide microarray. Among the antioxidant defense genes, five (*SOD1*, *PLK3*, *CLN8*, *XPA*, *HAO1*), whose expression was significantly altered by the action of fluoride ions and the exposure to SMF were normalized their expression was identified. We showed that fluoride ions cause oxidative stress, whereas exposure to SMF with a moderate induction can suppress their effects by normalizing the expression of the genes that are altered by fluoride. Our research may explain the molecular mechanisms of the influence of fluoride and SMF that are generated by permanent magnets on cells.

1. Introduction

For the last few decades, there has been increased interest in the influence of static magnetic fields (SMF) on the life processes of organisms as well as the potential risk that is associated with exposure to SMF. The continuous development of electrotechnical and electrical technologies and the decreasing prices of permanent magnets have initiated their widespread use not only in industry but also in consumer devices and diagnostic medical equipment [1,2].

Currently, neodymium iron boron magnets (NdFeB) are one of the most commonly used types of permanent magnets. Too little is known about the mechanisms of the influence of SMF on living organisms due to the complexity of this phenomenon as well as the degree and nature of their action [3,4,5]. Moreover, the modulation of the effect of other compounds and modification of their toxicity or cytoprotective action by SMFs are also not completely understood [6].

Fluoride is generally considered to be both an essential micronutrient for the proper formation of bones and teeth and as a toxic micronutrient that can cause fluorosis [7]. It occurs naturally in water at a concentration of 0.01–100 mg/l as well as in foods of a plant or animal origin. In the environment, glassworks, aluminum smelters, steel mills, phosphate fertilizer factories, brickyards and coal power stations are important sources of fluoride. In rural areas, the presence of fluoride in soil is the result of the excessive use of artificial fertilizers and pesticides. Apart from direct industrial, occupational and endemic exposure, more and more data have indicated an increase in the fluoride content in plants, fish and in dairy and meat products [8]. Moreover, there are also other sources of fluoride: fluoride prophylaxis of dental caries (tablets, mouthwash, toothpastes, dental lacquers, dental varnishes) and drugs that are used to treat osteoporosis for example [9]. It is believed that fluoride intake doubles every ten years.

Although the mechanisms of fluoride action have already been

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investigated in many studies, the results of these experiments do not fully explain these ones. It is believed that the toxicity of fluoride is associated with the processes of apoptosis and necrosis, inflammation and the induction of oxidative stress [10,11]. It was also confirmed that fluoride may induce changes at transcriptional level.

The effect of magnetic field on the human body has become the subject of many studies in light of the widespread exposure to it in the natural environment and in people occupationally exposed to SMF. Our research may lead to an improved understanding of molecular mechanisms of the combinatorial effect of SMF and fluoride on human cells. This combined exposure is typical of working conditions at electrolytic aluminum smelters, where workers are exposed to low- or moderate-intensity static magnetic fields [12]. Therefore, in the present study, we wanted to answer the questions of whether and in which direction SMF can modify the effect of fluoride on human cells as well as of whether these events are accompanied by the development of oxidative stress. Thus, the main aim of our study was to evaluate the simultaneous exposure of human cells to fluoride and the static magnetic fields that are generated by permanent magnets on the expression profile of the genes that are associated with the antioxidant defense system. In order to achieve our aims, we applied modern molecular biology techniques such as the oligonucleotide microarray.

2. Material and methods

2.1. Cell culture conditions

Normal human dermal fibroblasts (NHDF cell line) were obtained from Clonetics (CC-2511; San Diego, CA, USA) and routinely maintained in an FBM medium (Fibroblast Basal Medium, Lonza, Basel, Switzerland), which was supplemented with human fibroblast growth factor-basic (hFGF-B), insulin and gentamicin (FGM™ SingleQuots™; Lonza, Basel, Switzerland) at 37 °C in a 5% CO_2 incubator (Heraeus).

Both the number of cells and their viability were monitored by cell counting in a Countess TM Automated Cell Counter (Invitrogen, Carlsbad, CA, USA) after staining with 0.4% trypan blue. The experiment was performed on cells that were in the logarithmic phase of growth under conditions of ≥98% viability as assessed by trypan blue exclusion. For the experiments, NHDF cells were used at four to six passages.

2.2. Cytotoxicity

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) conversion method was used to determine whether fluoride (Sigma-Aldrich, St Louis, MO, USA) at concentrations between 0.02 and 1.00 mmol/l was toxic to the fibroblast cell cultures. The fluoride was prepared as a stock solution in phosphate buffered saline (PBS, Sigma-Aldrich, St Louis, MO, USA) and then diluted in the culture medium. The viability of the cells was evaluated after 24 h and 48 h of exposure to NaF. The effect of the fluoride ions on cell viability was evaluated in two independent experiments.

In the MTT assay, the ability of the cells to convert MTT (Sigma-Aldrich, St Louis, MO, USA) indicates mitochondrial activity and, as a result, cell viability. Normal human dermal fibroblasts were seeded into 96-well culture plates (Nunc, Wiesbaden, Germany) at a density of 5000 cells/well and were treated with fluoride ions for 24 and 48 h. MTT (0.25 mg/ml) was added to the medium for 3 h (37 °C) before the end of the experiment. After being washed with phosphate buffered saline (PBS), the cells were lysed in 100 µl of dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO, USA), which permitted the release of the blue reaction product – formazan. Absorbance at a wavelength of 540 nm was read on a Wallac 1420 VICTOR microplate reader (PerkinElmer, Waltham, MA, USA).

2.3. Exposure of NHDF cells to static magnetic fields

The control fibroblasts and the fibroblasts that had been treated with fluoride ions were then subjected to static magnetic fields. NaF was used at a concentration of 0.30 mmol/l because at a higher concentration, this compound induced a cytotoxic effect as was evidenced earlier in the cell viability assays.

To evaluate the effects of static magnetic fields, the control cells and the NHDF cells that had been treated with fluoride ions were placed in magnetic test chambers (patent P – 396639) [13]. The magnetic chambers that were used to culture the cells in a static magnetic field consisted of a ferromagnetic yoke, which constituted the bottom and cover of the chambers, and permanent magnets. The chambers were enclosed by lateral, front and back walls; the front wall was fitted with a window. The window dimensions corresponded to the lateral dimensions of a culture flask. Nonmagnetic distance plates determined the inner dimensions of the chambers, which were matched to the dimensions of a culture flask.

The design of these test chambers permits the uniform distribution of magnetic flux density over the measurement space of a flask. The flux density in the chambers was 0.65 T. The control culture chamber was not equipped with permanent magnets (steel was used instead) (flux density of 0.0 T). The cultures were maintained in the test chambers at 37 °C in a 5% CO^2 incubator (Heraeus) for 24 h.

Next, the cells were washed with PBS and the cell numbers were monitored by cell counting in a Countess TM Automated Cell Counter (Invitrogen, Carlsbad, CA, USA) after staining with 0.4% trypan blue. The cells were then pelleted and frozen at −70 °C for 24 h until RNA extraction.

2.4. Determination of reactive oxygen species (ROS) production

Intracellular ROS production was estimated via flow cytometry using 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA; Thermo Fisher Scientific, Waltham, MA USA). H_2DCF-DA is a cell permeable, nonfluorescent precursor of DCF. The acetate group of H_2DCF-DA from the molecule cleaves to the cells intracellular esterases to yield H_2DCF, which is then trapped within the cells. Intracellular ROS oxidize H_2DCF to form the highly fluorescent product – DCF.

After being trypsinized, the control cells and the NHDF cells that had been treated with NaF and exposed to SMF were centrifuged and then suspended in 0.1 µmol/l diluted H_2DCF-DA. The cells were incubated for 10 min in a 5% CO_2 incubator. For the positive control, the cells were first incubated with 5 mmol/l N-acetyl-L-cysteine (NAC, Sigma-Aldrich, St Louis, MO, USA) for 1 h at 37 °C and for the negative controls, the cells were first treated with 0.1 mmol/l H_2O_2 (Sigma-Aldrich, St Louis, MO, USA) for 20 min at 37 °C. Next, the cells were washed three times with DPBS (Dulbecco's phosphate buffered saline; Thermo Fisher Scientific, Waltham, MA USA) and then analyzed via flow cytometry (BD, FACS Aria II, USA) with the excitation and emission wavelengths at 488 and 525 nm, respectively. The fluorescence intensity of DCF represented the quantity of intracellular ROS.

2.5. RNA extraction

Total RNA was extracted using a TRIzol reagent (Invitrogen, Carlsbad, CA). The RNA extracts were treated with DNase I (RNeasy Mini Kit, Qiagen, Valencia, CA) according to the manufacturer's instructions. The quality of the extracts was determined electrophoretically using 0.9% agarose gel stained with ethidium bromide (Sigma-Aldrich, St. Louis, MO). The results were analyzed and recorded using the 1D Bas-Sys gel documentation system (Biotech-Fisher, Perth, Australia). The RNA concentration was determined using a GeneQuant II RNA/DNA spectrophotometer (Pharmacia Biotech, Cambridge, UK).