EFFECT OF SODIUM FLUORIDE ON THE CATALASE ACTIVITY IN THP-1 MACROPHAGES

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SUMMARY: Catalase, which catalyzes the conversion of hydrogen peroxide to water and molecular oxygen, is an antioxidative enzyme whose activity and cell content may change as a result of exposure to fluoride. In this paper we attempt to clarify the effect of fluoride on catalase activity, depending on NaF concentration and incubation time in THP-1 cell line macrophages. The obtained results proved the inhibitory effect of each of the used concentrations of NaF (0.5 mM, 1 mM, 5 mM) on enzyme activity. This effect could be explained by the interaction of fluoride ions with di- or trivalent metals located in the active site of antioxidative enzymes, which would lead to the inhibition of the enzyme—as in the case of catalase.

Keywords: Catalase; Fluoride; Macrophages; THP-1 cells.

INTRODUCTION

Catalase (CAT; EC 1.11.1.6), which catalyzes the conversion of hydrogen peroxide to water and molecular oxygen, is an antioxidative enzyme whose activity and cell content may change as a result of exposure to the fluoride ion (F).[1-4] Determining the exact impact of fluoride on the activity of this enzyme poses many difficulties, as the results of contradictory studies indicate.[5] Moreover, there is still little research clarifying the effect of this ion on the activity of antioxidative enzymes in human macrophages (which are the main source of reactive oxygen species (ROS) in organisms)—cells of critical importance for the inflammatory processes, depending on the concentration and time of exposure to fluoride. Therefore, in this paper we attempt to clarify the effect of NaF on CAT activity, depending on the concentration and incubation time in THP-1 cell line macrophages.

MATERIAL AND METHODS

Reagents: The following products from Sigma-Aldrich (Poland) were used: medium RPMI-1640, delipided bovine serum albumin (BSA), penicillin, streptomycin, glutamine, a reagent for protein determination using the Bradford method -N,N,N',N'-tetramethyl-p-phenylenediamine. From Purchem (Poland), sodium fluoride was obtained. The following products from POCH (Poland) were used: PBS (phosphate saline buffer, pH 7.4), H_2O_2. reagents for spectrophotometric measurement of the catalase activity.

THP-1 macrophage culture: THP-1 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Monocytes of the THP-1 cell line were cultured in RPMI-1640 medium enriched with 10% FBS, penicillin (100 U/mL),

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streptomycin (100 mg/mL) and glutamine (2 mM/mL). The cultivation was conducted at 37ºC in an environment of 5% CO_2 and 90% humidity in an incubator manufactured by Assab Kebo Lab (Sweden). Minor laboratory equipment came from Sarstedt (Germany) and Becton-Dickinson (USA). Viability of the cells used was more than 95%.[6] The cells were suspended in warm (36ºC) RPMI -1640, without FBS. Cultivation was carried out in six-well incubation plates, with 4 million culture cells in a single well. To each well a 100 nM/L PMA was added and incubation was performed for 24 hours. The obtained macrophage cells were adherent.[7] After the incubation cells were collected with a rubber scraper. The suspension was centrifuged (800 g/10 min/4ºC). The resulting precipitate was suspended in PBS. Suspension was frozen at –80ºC.[8]

Experimental conditions and CAT activity determination: Before the experiment frozen macrophages were thawed and the cells suspension was centrifuged (800 g/ 10 min/4ºC). For protein determination, 30 µL of the homogenate was collected. The remaining part of the supernatant was removed with a pipette and the cell pellet was suspended in the NaF solution respectively 0.5 mM, 1 mM, or 5 mM. The effect of each NaF concentration on the CAT activity in THP-1 macrophages was examined over a range of incubation time (10, 30, or 60 min). Afterwards the NaF solution was centrifuged and the cell pellet was washed twice with PBS and centrifuged (800 g/10 min/4ºC). THP-1 macrophages incubated in PBS (without NaF) were used for control. In the obtained precipitate the CAT activity was estimated. CAT activity was determined by the method proposed by Aebi.[9] Enzyme activity was measured using a spectrophotometer using a Perkin Elmer Lambda 400 UV/VIS spectrophotometer (USA). All obtained results were calculated by protein content in samples. The protein content was determined using the Bradford method.[10]

Statistical calculations: For statistical analysis nonparametric tests were used. For related variables a T-Tukey and Friedman ANOVA tests were used. Statistically significant results were reanalyzed by a Wilcoxon matched-pairs test. All calculations were performed using Statistica 7.0 software (Statsoft, Poland). p values of ≤0.05 were considered statistically significant. Results were expressed as mean value±SD.

RESULTS

The multifactorial analysis (Tukey test) indicated both the NaF concentration and the incubation time significantly influenced the CAT activity ($p<0.05$). According to Friedman ANOVA, NaF inhibits THP-1 macrophage CAT activity in a time-related manner.

NaF used at the concentration of 0.5 mM significantly inhibited CAT activity after 30 min ($n=6$; $p<0.05$) and 60 min ($n=6$; $p<0.05$) of incubation if compared to control (Wilcoxon Match-pairs test). After 10 minutes of incubation only a downward tendency was observed (Figure 1).

1 mM NaF also exerted an inhibitory effect on the CAT activity. The enzyme activity decreased significantly after 10 min ($n=6$; $p<0.05$) and 60 min of

incubation (n=6; p<0.05) if compared to control (Wilcoxon Match-Pairs test). After 30 min of incubation only a downward trend was observed.(Figure 2).

Incubation with 5 mM NaF resulted in CAT activity inhibition. Enzyme activity decreased significantly after 10 min (n=6; p<0.05), 30 min (n=6; p<0.05) and 60 min (n=6; p<0.05) of incubation time *versus* control (Figure 3).

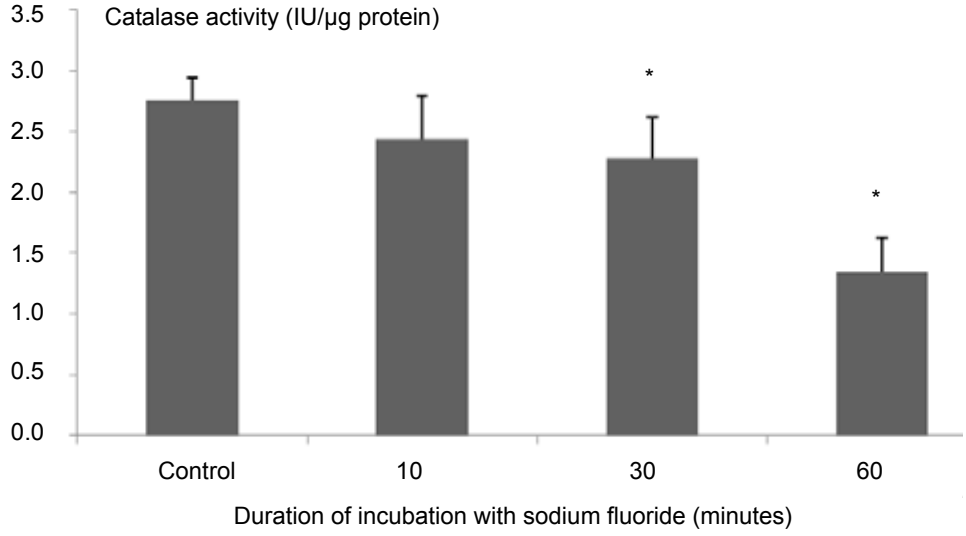

Figure 1. Influence of 0.5 mM NaF on catalase activity in THP-1 macrophages. The enzyme activity was estimated after 30 and 60 minutes of incubation with NaF. Macrophages not exposed to NaF were used as a control. *p <0.05 versus control.

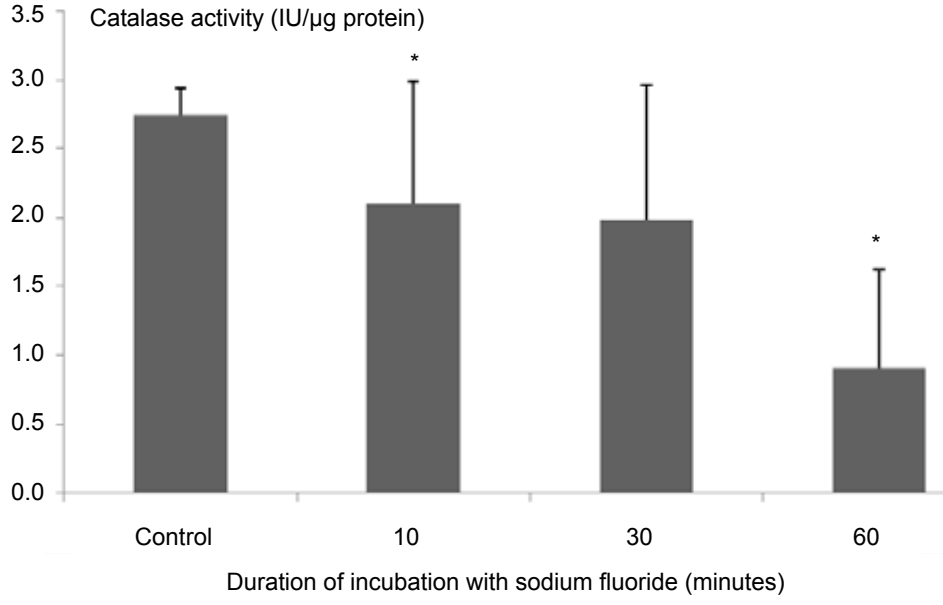

Figure 2. Influence of 1 mM (B) NaF on catalase activity in THP-1 macrophages. The enzyme activity was estimated after 30 and 60 minutes of incubation with NaF. Macrophages not exposed to NaF were used as a control. *p <0.05 versus control.

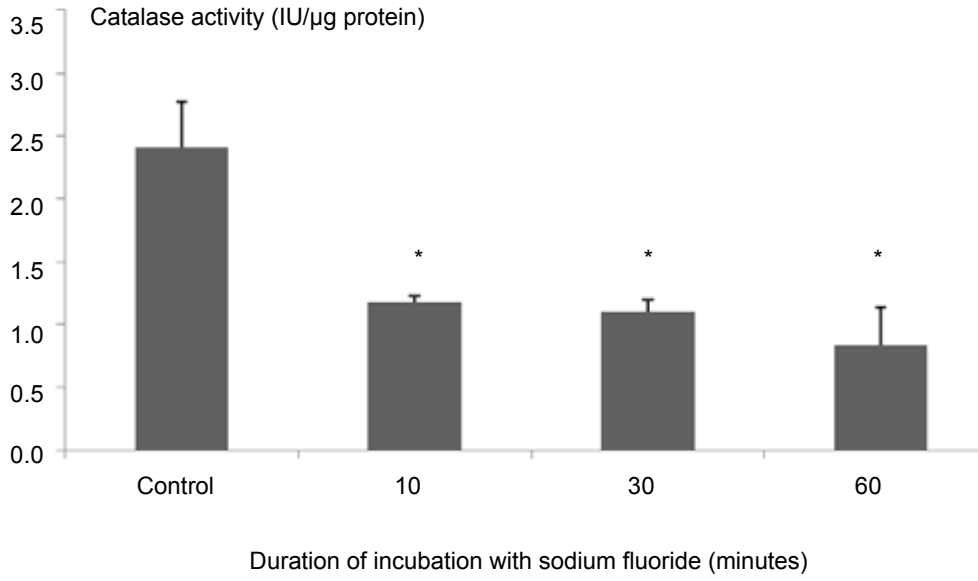

Figure 3. Influence of 5 mM NaF on catalase activity in THP-1 macrophages. The enzyme activity was estimated after 30 and 60 minutes of incubation with NaF. Macrophages not exposed to NaF were used as a control. *p <0.05 versus control.

Additionally, the influence of each NaF concentration after 10, 30, and 60 min was compared. After 10 min of incubation the CAT activity was significantly higher in macrophages incubated with 0.5 mM NaF in comparison to macrophages incubated with 5 mM (n=6; p<0.05). There were no significant differences between cells incubated with 0.5 mM and 1 mM, and between cells incubated with 1 mM and 5 mM NaF (Figure 4).

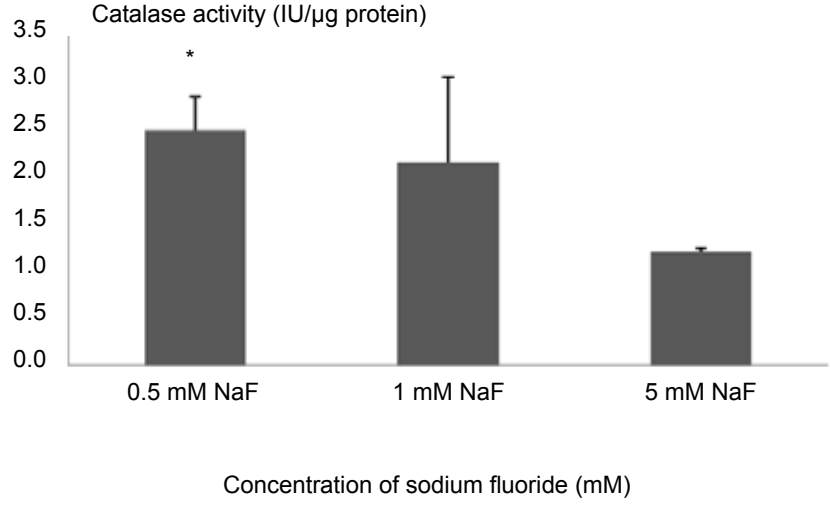

Figure 4. Influence of different concentrations of NaF (0.5mM NaF, 1mM NaF, 5mM NaF) on catalase activity in THP-1 macrophages after 10 minutes of incubation. *p<0.05 for the 0.5 mM NaF group compared to the 5 mM NaF group.

CAT activity after 30 min of incubation was significantly decreased in macrophages cultured with 5 mM NaF in comparison to both 0.5 mM NaF (n=6, $p<0.05$) and 1 mM NaF (n=6, $p<0.05$, Figure 5).

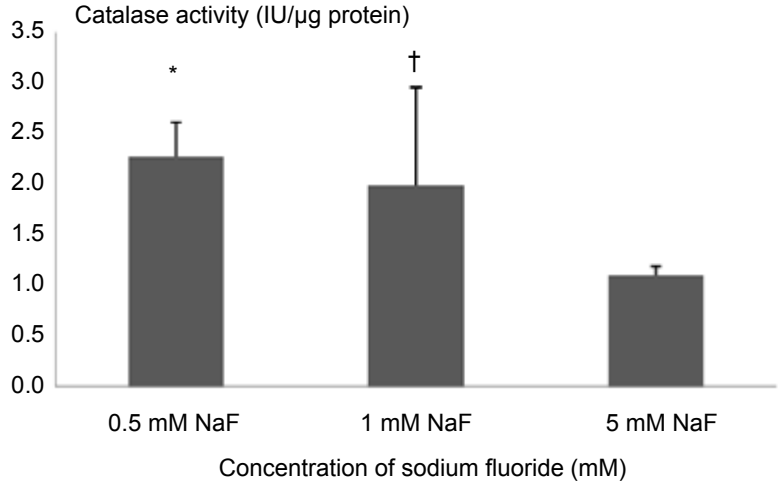

Figure 5. Influence of different concentrations of NaF (0.5mM NaF, 1mM NaF, 5mM NaF) on catalase activity in THP-1 macrophages after 30 minutes of incubation. *$p<0.05$ for the 0.5 mM NaF group compared to the 5 mM NaF group, †$p<0.05$ for the 1 mM NaF group compared to the 5 mM NaF group

After 60 min a significant difference was observed only in cells incubated with 0.5 mM NaF in comparison to those cultured with 5 mM NaF (n=6, $p<0.05$, Figure 6).

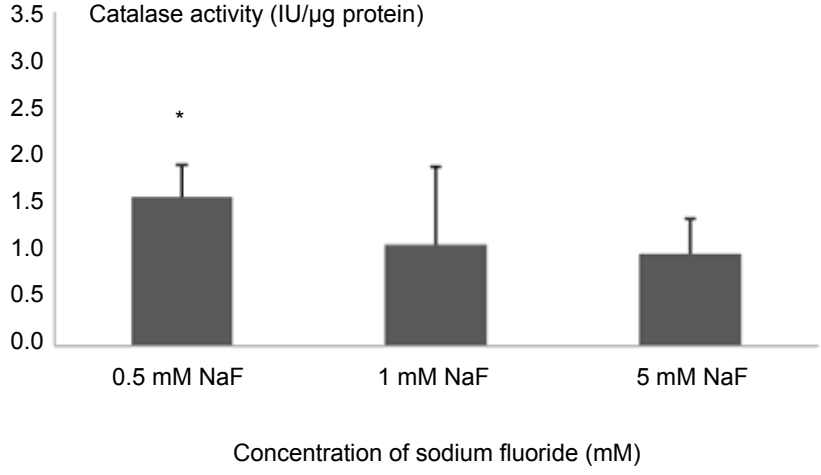

Figure 6. Influence of different concentrations of NaF (0.5mM NaF, 1mM NaF, 5mM NaF) on catalase activity in THP-1 macrophages after 60 minutes of incubation. *$p<0.05$ for the 0.5 mM NaF group compared to the 5 mM NaF group.

DISCUSSION

The fluoride ion (F), of the element fluorine, is very common and can have negative effects on the human body.[11] Excessive exposure to F may result in

fluorosis, which might take an acute or chronic form.[12-14] Fluorosis is a significant problem, particularly in developing countries where drinking water is the main source of F.[12] Excessive exposure to F can lead to severe and irreversible changes, e.g., in the nervous, skeletal, muscular, and digestive systems. One of the possible mechanisms of the toxic effect of F on tissues of organisms is its involvement in the pathogenesis of oxidative stress,[15,16] characterized by an imbalance between the processes of production and degradation of reactive oxygen species (ROS).[17] Living organisms have a range of antioxidants whose role is to protect the cells against the harmful effects of ROS. Among them, a particularly important role is fulfilled by antioxidative enzymes, including CAT which is present in almost all aerobic organisms.[18,19] This enzyme is located within peroxisomes and catalyzes the decomposition of H_2O_2.[20,21]

In recent years, more and more attention is being paid to issues related to the participation of F in the pathogenesis of oxidative stress. A number of reports indicate a bidirectional spread of this phenomenon. On one hand, F can lead to an increased production of ROS. On the other hand, it serves as an effector of many enzymes, including antioxidative types, which are of key importance in preventing the negative effects of ROS.[22-24] Despite the continuous proliferation of publications on the involvement of F in oxidation and reduction processes, available results are often contradictory.

The inhibitory effect of F on CAT activity is reported in people living in endemic fluorosis areas as well as in animal models exposed to F.[5,15,25-27] The inhibitory effects of NaF on CAT activity within erythrocytes, liver, and kidney in a rabbit model were proven by Ranjan.[28] The same effects of F on CAT activity in various organs are also described in studies conducted on rats by Bharti and Srivastava,[29] Błaszczyk et al.,[30] Yamaguti,[31] on broilers by Liu et al.[32] and on mice by Vani and Reddy,[33] Patel and Chinoy,[34] Sun et al.[35] and Dierickx and De Beer.[36]

This approach to the effect of fluoride ions on CAT activity seems to be described most frequently, as well as in the human model. Many authors prove the inhibitory effect of NaF on CAT activity within human erythrocytes in people living in India[4,26] and China.[37]

In our experiment, we also proved the inhibitory effect of NaF on CAT activity, in this case, in THP-1 macrophages. Each of the used concentrations of NaF (0.5 mM NaF, 1 mM NaF, and 5 mM NaF) resulted in the inhibition of enzyme activity. The enzyme activity decreased as the concentration of NaF was increased and the incubation time prolonged. This effect, observed in our experiment as well as in the aforementioned studies, could be explained by the interaction of F with di- or trivalent metals located in the active site of antioxidative enzymes, which would lead to the inhibition of the enzyme—as in the case of CAT.[31,38]

In contrast to the results obtained in this experiment, there are reports which prove a lack of effect of F on CAT activity in rats erythrocytes[39,40] or an increase in CAT activity after exposure to F in both plant[41] and animal models,[42,43] as well as in studies on humans.[44]

The examples presented above prove that determining the exact effect of F on the activity of CAT poses many difficulties, despite the many tests conducted time and again on the same enzymes. The results of experiments are often inconsistent,[45-47] and the manner in which F affects the activity of CAT seems to depend on many factors. The observed ambiguities may arise from the diversity of the species and the age of the examined organisms. The fluoride dose, type of exposure, duration of exposure and the sensitivity of the tissue to fluoride are listed as possible causes of these discrepancies.[48]

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