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ORIGINAL ARTICLE

Induction of apoptosis in human acute monocytic leukemia (THP-1) cells with NaF and the related mechanisms

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Summary

Purpose: *Acute monocytic leukemia remains a big challenge, and there are a series of non-specific esterases which can be inhibited by sodium fluoride (NaF) in mononuclear cells, providing insights into the leukemia targeted therapy. In this study, the apoptotic effect of NaF with human mononuclear leukemia THP-1 cells and the inhibition of a-naphthol acetate esterase (a-NAE) activity, and also the potentially underlying mechanisms were investigated.*

Methods: *THP-1 cells were cultured with different concentrations of NaF (0, 0.5, 1, 2, 4, 8 mM) for 24, 48 and 72 hrs. The a-NAE staining and chromogenic method were used to detect the activation of a-NAE. The 3-(4, 5-dimethylthiazol-2-yl)-2), 5-diphenyltetrazolium bromide (MTT) assay was used to detect the antitumor effect of NaF on THP-1 cells in vitro. Flow cytometry was used to observe the apoptotic ratio following treatment with NaF in THP-1 cells. The mRNA levels of mammalian target of Bcl-2 and Bax were detected pre and post-NaF treatment using reverse transcription polymerase chain reaction (RT-PCR).*

Results: *The generation of depression effects of THP-1 cells cultured in vitro were detected using MTT technology, which revealed a dose- and time-dependent association. After 24hrs of exposure to NaF at greater than 1mmol/L, typical apoptotic changes were observed, accompanied by the a-NAE positive reaction and decreased intensity. The IC_{50} was 4 mmol/L at 24hrs. Flow cytometric analysis indicated that treatment with NaF at concentrations of 2, 4 and 8 mmol/L increased the apoptotic rate of THP-1 cells. RT-PCR indicated that NaF upregulated the gene expression of Bax and downregulated the expression of Bcl-2.*

Conclusion: *NaF inhibited the proliferation of THP-1 cells and the activation of a-NAE by adversely regulating the expression of Bax and Bcl-2.*

Key words: *acute monocytic leukemia, a-NAE, apoptosis, Bax, Bcl-2, THP-1 cells*

Introduction

Leukemia, a malignant hematopoietic disease, is a serious threat to human life and health. The incidence of leukemia was 5.17/100,000 in China in 2015, when it was the most common tumor in children and adults less than 35 years of age [1,2]. Acute myeloid leukemia (AML) is the most common type of leukemia, and the incidence of acute monocytic leukemia is highest [2]. Acute monocytic leukemia with high tumor burden, extensive extramedullary infiltration and high drug resistance, has a poor prognosis [3]. Therefore, selection of new effective drugs has significant value for the treatment of this disease.

Esterases are hydrolases capable of hydrolyzing the ester bond to produce an acid and an alcohol. Esterases can affect enzymes, signaling proteins and transcription factors' pathway activity by regulating protein phosphorylation and dephosphorylation [4,5], and then can play a role in the metabolism and energy metabolism , regulation of gene expression and cell signaling [6-8]. Previous evidence has concluded that there are a

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series of non-specific esterase isozymes in monocytes whose activity can be inhibited by NaF [6]. This feature is different from other mononuclear blood cells.

Fluorine is a non-metalic element with lively chemical properties. Fluorine can affect the activity of various enzymes such as enolases, cholinesterases, adenosine triphosphate and play a part in cell response [9]. On the one hand, fluoride is one of the trace elements necessary for the body. A certain amount of fluoride can promote the growth of bones and teeth, stimulate hematopoiesis and play a part in the facial growth and mental development [10]. On the other hand, it is reported that fluoride can affect the respiratory system [11], circulatory system [12] and other systems. Some studies concluded that low concentrations of fluoride can induce transformation of HL-60 leukemia cells into myeloid differentiation and promote their proliferation [13], while high concentrations of sodium fluoride were able to induce apoptosis [14].

Targeted therapy is a type of treatment that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth. For example, retinoic acid (ATRA) induces acute promyelocytic leukemia (M3) cell differentiation by degrading PML/RARα fusion protein [15]. Imatinib can degrade the activity of tyrosine kinase by specificity-binding of imatinib BCR/ABL tyrosine kinase domain, and then induce chronic myeloid leukemia cells apoptosis; rituximab can specifically kill lymphoma cells by binding with the CD20 of B lymphocytes.

As previously mentioned [10-12], protein phosphorylation and dephosphorylation conditioning systems are composed of phosphatases and protein kinases. Tyrosine kinase inhibition can achieve the purpose of targeted therapy of leukemia (such as targeted therapy imatinib in chronic myeloid leukemia). So, can esterase inhibition achieve the purpose of targeted therapy in leukemia? Thus, we wonder, whether fluoride could induce apoptosis of monocytic leukemia cells by esterase inhibition, so as to achieve the purpose of targeted therapy of this disease.

Methods

Materials

Human monocytic leukemia cells (THP-1, ATCC TIB-202), firstly described by Auwerx [16], were purchased from Xiangya Medical College Cell Center (Changsha, Hunan, China). NaF, a-acetic ester Chennai, solid blue B salt and α-naphthol (AR) were purchased from Sinopharm (Shanghai, China). RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Gibco (California, USA). Penicillin, streptomycin and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (California, USA). FITC Annexin V/propidium iodide (PI) were purchased from Becton Dickinson Co (New Jersey, USA). Trizol and M-MLV inverse transcription kit were purchased from Invitrogen Co (California, USA) and 2×Tap Master Mix and DNA Mark I were purchased from Shanghai Sangon Biotech (Shanghai, China).

Cell culture and MTT test

THP-1 human monocytic leukemia cells were cultured in RPMI 1640 supplemented with 20% FBS and penicillin- streptomycin of 0.5% in a humidified incubator with 5% CO2 at 37°C. Exponentially growing THP-1 cells were seeded at 3-4×10^4 cells per well in 96-well plate and treated with varying concentrations (0, 0.5, 1, 2, 4, and 8 mM) of NaF. After 24, 48 and 72 hrs of exposure, the general viability of cultured cells was determined by MTT assay.

Treatment with drugs and morphological observations

Logarithmically growing THP-1 cells were seeded in a 6-well plate at a concentration of 1×10^6/ml and treated with varying concentrations of NaF as described for 24 hrs. After exposure, cells were observed under an inverted phase-contrast microscope and under microscope after Wright-Giemsa staining, respectively.

Qualitative measurement of a-NAE activity by cytochemical staining

At the end of treatment, the cells in 6-well plates were harvested, transferred into sample tubes and centrifuged (1000 rpm for 5 min) to remove supernatants and the remaining cell suspension was made to smears. After being fixed with formaldehyde, the smears were incubated in solution at 37°C (solution formulation: 1/15M KH2PO4 20 ml; 1/15M Na2HPO4 80 ml; 50% acetone 2 ml; a-NAE 20 mg; Solid blue B salt 100 mg). After 1 hr of incubation, the smears were observed under a microscope.

Quantitative measurement of a-NAE activity by colorimetry

The principles and procedures are described previously in this study. Briefly, at the end of treatment, the cells were harvested, transferred into sample tubes (1×10^6cells per tube), and centrifuged (2500 rpm for 5 min) to remove supernatants, added with 300 μl double-distilled water (ddH_2O), sonicated intermittently, and then centrifuged to collect supernatant. 100 μL of ddH_2O as Zero tube, 2 mM a-naphthol as standard and sample solution were added to tube respectively, then added 0.5 ml 2 mM a-acetate ester Chennaisubstrate solution as substrate solution and incubated at 37°C for 20 min. And then, l mL 1.75 M acetic acid as stop solution and 1 mL 1 mM solid blue B salt as color liquid were added into the tubes. After incubation at 37°C for 20 min, the absorbance values were measured by UV spectrophotometer at 520 nm wavelength, 0.5 cm optical path (Formula: α-NAE (U/10^^{9}cell) = sample tube A / standard tube A × 0.2 ÷ 20 × 3 × 1000).

Flow cytometry analysis of apoptosis

Cells were treated with various concentrations of NaF for 24 hrs, as previously mentioned, were collected and harvested by centrifugation at 1000 rpm for 5 min. Cell pellets were washed twice with precooled PBS and resuspended in PBS at a cell concentration of 1×10^6/ml. Cell suspension (100 µL) was fixed with 5 µl Annexin V-FITC and 5 µl PI for 20 min at room temperature in the dark, and then added 400 µL of 1×buffer. A FACS Calibur flow cytometer (Becton Dickenson, California, USA) was used to analyze the level of apoptotic cells according manufacturer's instructions.

Semi-quantitative RT-PCR analysis

Total RNA was extracted from THP-1 cells using the TRIzol reagent (Invitrogen, California, USA). The concentration and purity of RNA were assessed spectrophotometrically (Thermo, Massachusetts, USA) at 260 and 280 nm, and the A260/A280 ratio was found to be greater than 1.8. For the RT reaction, total RNA (2 µg) was primed with Oligo (dT) and reverse-transcribed into cDNA with 200 units of M-MLV reverse transcriptase from Invitrogen (California, USA), according to manufacturer's instructions, in a final volume of 20 µL. The mixture was incubated at 37°C for 50 min and 70°C for 15 min. The generated cDNA was used for the semi-quantitative RT-PCR analysis to assess mRNA expression, and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as internal control. A 25-µL PCR mixture contained 1.5 µL of the RT reaction, 5 pmol of each primer and 12.5 µL 2×Taq Master Mix. PCR reactions were performed in a GeneAmp PCR System model (Applied Biosystems, Massachusetts, USA). The primers used are listed in Table 1 designed according to references [17]. Amplification of the expected single PCR product was confirmed using 1.5% agarose gels stained with ethidium bromide.

Statistics

All data derived from at least three independent experiments. Statistical analyses were conducted using SPSS software (Version 17.0). Values were presented as mean ± SD. Significant differences between the groups were determined using the variance analysis, homogeneity of variance using least significant difference (LSD) method and heterogeneity of variance with Tamhane's T2 [18]. For bivariate correlation analysis, if the data were normally distributed, Pearson correlation analysis was used, otherwise the Spearman rank correlation test was used. Statistical significance was set at $p<0.05$.

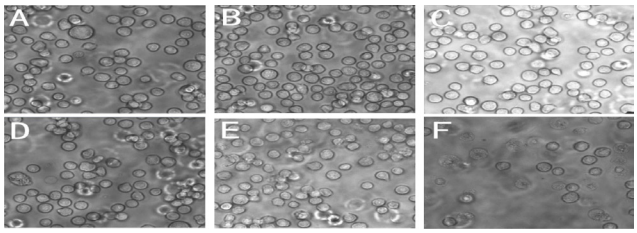

Figure 1. Light microscope photographs of NaF-treated THP-1 cells. Cells were treated with **(A)** with PBS or **(B-F)** with 0.5, 1, 2, 4, and 8 mmol/L of NaF for 24 hrs. There were no significant changes in cell morphology between **B, C** and **A**. Photomicrographs **(D-F)** show representative morphological changes that were observed under a phase-contrast microscope (400×).

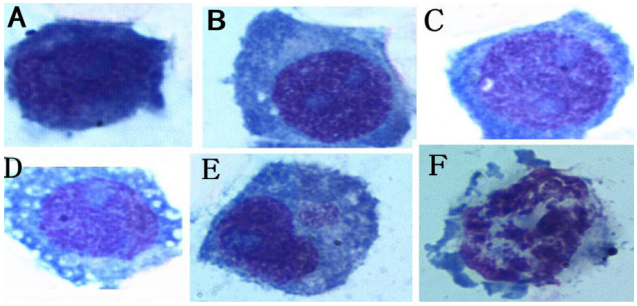

Figure 2. Wright staining images of NaF-treated THP-1 cells. Cells were treated with with PBS **(A)** or with 0.5, 1, 2, 4, and 8 mmol/L of NaF for 24 hrs **(B-F)**. There were no significant changes in cell morphology between **B, C** and **A**. Typical apoptotic changes were observed in **D-F** cells including vacuolar degeneration, chromatin condensation, and formation of apoptotic bodies (1000×).

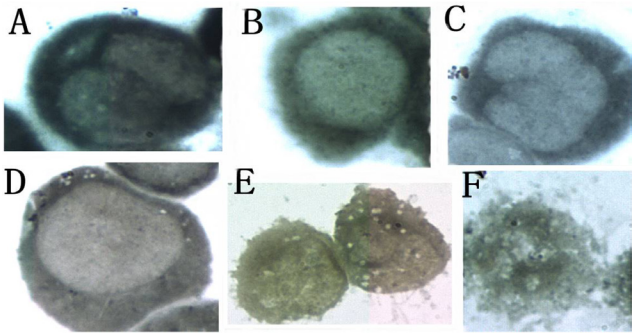

Figure 3. α-NAE staining images of NaF-treated THP-1 cells. Cells were treated with with PBS **(A)** or with 0.5, 1, 2, 4, and 8 mmol/L of NaF for 24 hrs **(B-F)**. The results show that with increasing concentration of NaF, the intensity of the α-NAE positivity gradually decreased.

Results

The morphological changes induced by NaF

Microscopic analysis of cells treated with NaF indicated that they had undergone gross morphological changes. The control group and the low-concentration group (NaF<1 mmol/L) had no significant change in cell morphology. After 24 hrs of exposure to NaF at >1mmol/L, typical apoptotic changes were observed, including cell shrinkage, vague cell outline, vacuolar degeneration, chromatin condensation, and formation of apoptotic bodies (Figure 1). Similar results were also observed by Wright staining (Figure 2).

Effect of NaF treatment on a-NAE activity

α-NAE staining was used to qualitatively observe a-NAE activity of THP-1 cells under optical microscope. The results show that with increasing concentration of NaF, the positive rate of THP-1 cells and the intensity gradually decreased, which indicated that NaF could inhibit the a-NAE activity of THP-1 cells in a dose-dependent manner (Figure 3).

We also used colorimetric analysis to quantitatively detect the a-NAE activity of THP-1 cells, and the results showed that there was a negative correlation between the a-NAE activity of THP-1 cells and the concentration of NaF (correlation coefficient r=-0.865, p=0.026), which indicated that with increasing concentration of NaF, the intensity of the positive of a-NAE activity gradually decreased. In addition, we performed univariate analysis of variance between the different concentrations of NaF, and the results showed that there were significant statistical differences between different concentrations of NaF (p<0.05;Table 1 and Figure 4).

Anti-proliferative effect of NaF in THP-1 cells

The effect of NaF treatment on THP-1 cells was evaluated using MTT assay. As shown in Table 2, Figure 5 and Figure 6, when the concentration of NaF was 0.5mmol/L, the inhibition rate (IR) at different times (24, 48 and 72 hrs) of exposure groups were negative, which indicated that low concentrations (0.5mmol/L) of NaF had no significant effect; conversely, it could promote the cells growth slightly. When the time was fixed, the difference of inhibition rate between various concentrations was statistically significant (p<0.05). The correlation coefficient r between concentration and inhibition rate of different times of exposure groups (24, 48, 72 hrs) were 0.986 (p=0.002), 0.953 (p=0.012) and R=0.912 (p=0.031), meaning that there was positive correlation between the concentration and the inhibition rate (Table 3). These results indicated

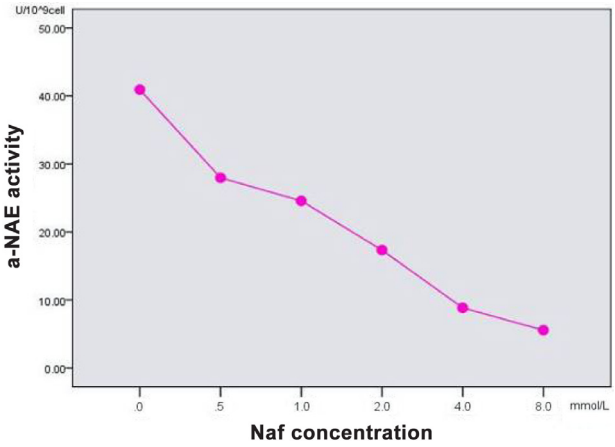

Figure 4. Effect of NaF treatment on α-NAE activity. With the increase of NaF concentration, the α-NAE positive reaction intensity and positive rate decreased (p<0.05).

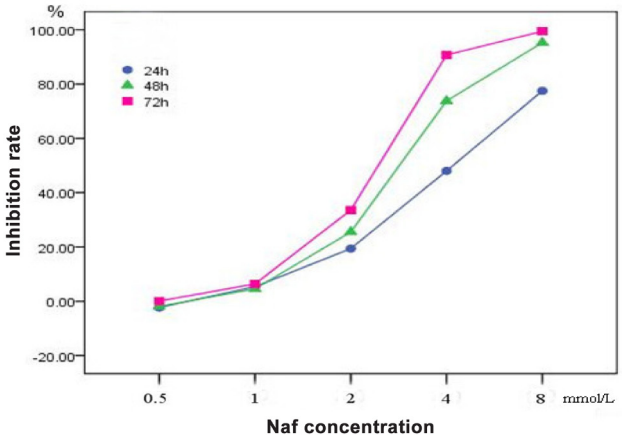

Figure 5. Anti-proliferative effect of NaF in THP-1 cells. Cells were incubated with 0.5, 1, 2, 4, and 8 mmol/L of NaF for 24, 48 and 72 hrs and cell survival was measured by the MTT assay. When the time was fixed, the difference of inhibition rate between various concentrations was statistically significant (p<0.05). These results indicated that NaF inhibited cell proliferation in an apparent dose-dependent manner. On the contrary, when the concentration of NaF was fixed at 0.5 mmol/L or 1 mmol/L, the difference of the inhibition rate of different times (24, 48, 72 hrs) was not statistically significant (p=0.385 or 0.596, respectively). In other concentrations of NaF, the inhibition rate increased with increase of time (p <0.05).

Table 1. Primers used for RT-PCR analysis of the GAPDH, Bax, Bcl-2 genes

Gene PCR	*Primer sequence*	*Annealing temperature (°C)*
GAPDH	Forward CAAGGTCATCCATGACAACTTTG	64
	Reverse GTCCACCACCCTGTTGCTGTAG	
Bax	Forward TGGCAGCTGACATGTTTTCTGAC	64
	Reverse TCACCCAACCACCCTGGTCTT	
Bcl-2	Forward TTCTTTGAGTTCGGTGGGGTC	59
	Reverse TGCATATTTGTTTGGGGCAGG	

that NaF inhibited cell proliferation in an apparent dose-dependent manner. When the concentration of NaF was 0.5mmol/L or 1mmol/L, the difference of the inhibition rate of different times (24, 48, 72 hrs) of exposure groups was not statistically significant (p=0.385 or 0.596, respectively). Although the exposure time was increased, the inhibition rate was not increased. This indicated again that low concentrations of NaF had no significant effect. In other concentrations of NaF, the inhibition rate increased with increasing time (p<0.05). The concentration required for 50% growth inhibition (IC_{50}) after 24, 48, and 72 hrs of treatment was determined to be 4 mmol/L, 2.7 mmol/L, and 2.4 mmol/L, suggesting that NaF inhibited cell proliferation in a time-dependent manner.

Flow cytometry analysis of apoptosis

In order to investigate whether NaF induces apoptosis at the indicated concentrations of NaF (0, 0.5, 1, 2, 4, and 8 mmol/L) in THP-1 cells for 24 hrs, induction of apoptosis in NaF-treated THP-1 cells was further analyzed by flow-cytometric (Figure 7). The apoptosis rates were 11.53±0.97%, 12.28±1.78%, 16.11±2.46%, 31.56±3.09%, 42.37±3.51% and 69.52±7.06%, respectively. The apoptosis rate was no significantly different (p=0.778 or 0.097) when the concentrations were at 0.5 mmol/L and 1 mmol/L, which indicated that low concentrations (0-1 mmol/L) of NaF had no significant effect. The apoptosis rate increased with

Table 2. Effect of NaF treatment on a-NAE activity (mean±SD)

Concentration of NaF (mmol/L)	*a-NAE activation (U/10⁹cell)*
0	40.92±2.63*
0,5	27.96±1.29*
1	24.57±1.37*
2	17.33±1.82*
4	8.84±0.23*
8	5.56±0.21*

*pairwise comparison of each concentration, p<0.05.

Table 3. Anti-proliferative effect of NaF in THP-1 cells (mean±SD)

Concentration of NaF (mmol/L)	*24 hrs IR%*	*48 hrs IR%*	*72 hrs IR%*
0,5	-2.33±3.14	-1.9±2.04	-0.08±3.35
1	5.38±1.57	4.57±2.78	6.37±4.14
2	19.40±2.69	25.50±5.74	33.58±6.24
4	48.00±8.69	73.85±4.94	90.72±1.88
8	77.50±7.56	95.25±2.82	99.46±0.36

IR: inhibition rate

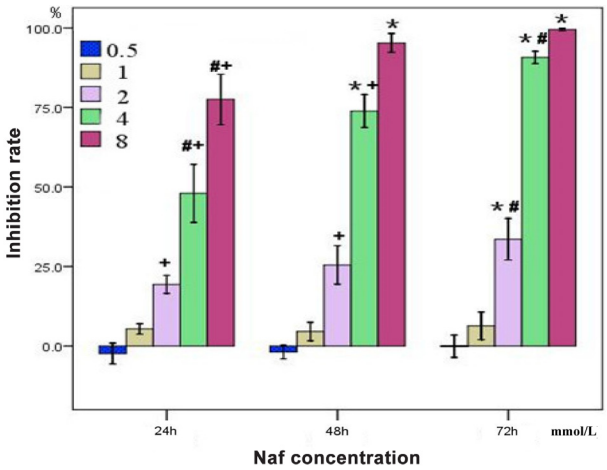

Figure 6. Anti-proliferative effect of NaF in THP-1 cells at three time points (24, 48 and 72 hrs), showing the differences of IR beweem 0.5, 1, 2, 4, 8 mmol/L groups. When the concentration of NaF was 0.5mmol/L or 1 mmol/L, the difference of IR of different times (24, 48 and 72 hrs) of exposure groups was not statistically significant (p=0.385 or 0.596, respectively). This indicated again that low concentrations of NaF had no significant effect (*p<0.05 vs 24 hrs; #p<0.05 vs 48 hrs;+p<0.05 vs 72 hrs).

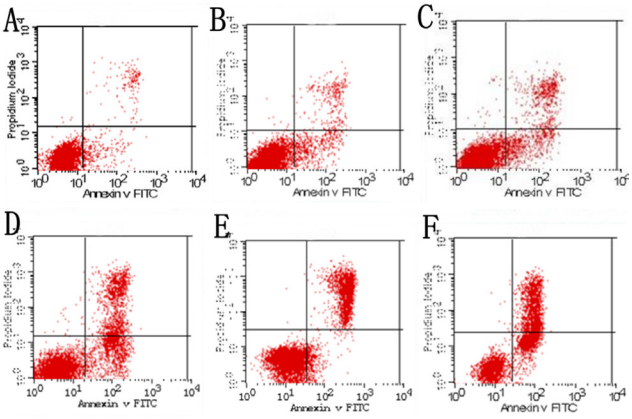

Figure 7. Apoptosis induction of NaF in TPH-1 cells for 24 hrs. Cells were treated with with PBS (A) or with 0.5, 1, 2, 4, and 8 mmol/L of NaF for 24hrs (B-F). The apoptosis rates were: 11.53±0.97%, 12.28±1.78%, 16.11±2.46%, 31.56±3.09%, 42.37±3.51% and 69.52±7.06%, respectively. The apoptosis rate had no significant difference (p=0.778 or 0.097) when concentrations were at 0.5 mmol/L and 1mmol/L, which indicates that low concentrations (0-1mmol/L) of NaF had no significant effect. The apoptosis rate increased with the cumulative NaF concentrations, which suggests that NaF-induced apoptosis in THP-1 cells was dose-dependent at concentrations ranging from 2 to 8 mmol/L.

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Bcl-2 and Bax mRNA levels during differentiation

Bax and Bcl-2 mRNA levels were represented by the ratios of Bax/GAPDH and Bcl-2/GAPDH. The Bax and Bcl-2 mRNAs were unchanged after 0-1 mmol/L NaF treatment (p>0.05). The Bax mRNA was gradually increased positively proportionally to the NaF concentrations, and Bcl-2 mRNA was decreased at concentrations of NaF ranging from 2 to 8mmol/L (p<0.05; Figures 8 and 9).

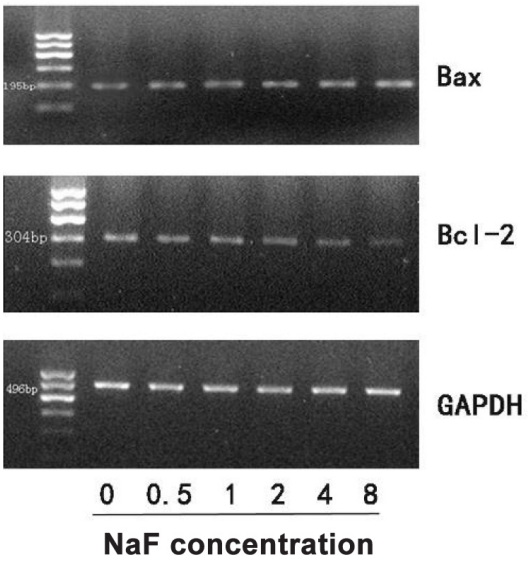

Figure 8. The Bax and Bcl-2 mRNA levels in THP-1 cells by semi-quantitative RT-PCR. Cells were exposed to various concentrations of NaF for 24 hrs. Bax and Bcl-2 mRNA levels were represented by the ratios of Bax/GAPDH and Bcl-2/GAPDH. The ratios of Bax (0-8 mmol/L): 0.65±0.07, 0.71±0.06, 0.71±0.04, 0.79±0.07, 0.89±0.01, 0.99±0.08. The ratios of Bcl-2 (0-8 mmol/L): 0.58±0.08, 0.56±0.06, 0.58±0.06, 0.48±0.02, 0.30±0.04, 0.17±0.03.

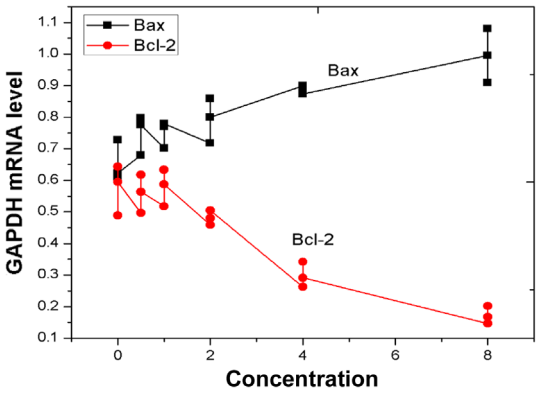

Figure 9. The Bax (left) and Bcl-2 (right) mRNA levels in THP-1 cells after treatment by various concentrations of NaF for 24 hrs. Bax and Bcl-2 mRNA levels were represented by the ratios of Bax/GAPDH and Bcl-2/GAPDH. The Bax and Bcl-2 mRNA were unchanged after 0-1 mmol/L NaF treatment (p>0.05). The Bax mRNA gradually increased proportionally to the NaF concentrations, and Bcl-2 mRNA decreased at concentrations of NaF ranging from 2 to 8 mmol/L (p <0.05).

Discussion

Fluoride is one of the trace elements necessary for the body. A certain amount of fluoride can promote the growth of bones and teeth, stimulates hematopoiesis, and plays a part in interfacial growth and mental development. However, excess fluoride can cause damage to the human organism. Fluoride can affect ATP enzyme, phosphatase activity and G-protein signal transduction pathways, triggering a series of cellular responses [11,19]. This study has shown that NaF could inhibit protein synthesis, dangerously damaging DNA, even inducing apoptosis in leukemia cells. Chemotherapy-induced apoptosis is one of the anti-leukemia mechanisms.

In this study, we treated THP-1 cells with various concentrations for 24 hrs, and we found that cell morphology changed, including cell shrinkage, vague cell outline, vacuolar degeneration, chromatin condensation, and formation of apoptotic bodies, which showed the typical apoptotic morphological changes. This morphological changes initially indicated that NaF induced apoptosis in THP-1 cells. In order to investigate the apoptosis induction of NaF in THP-1 cells, we evaluated the proliferation inhibition rate by using modified MTT assay. Compared with the conventional MTT assay, modified MTT assay eliminates the centrifugation step and streamline operations while improving accuracy, especially for the detection of proliferation inhibition rate of cell in suspension. The results showed that the inhibition rate was negative at low concentration (0.5mmol/L), illustrating that at low concentrations (<0.5mmol/L) fluoride acts as a relatively weak stimulator of cell proliferation. It seems that at relatively low levels, NaF has a stimulatory effect on DNA and protein biosynthesis [20]. With increasing concentration, the inhibition rate was gradually increased at concentrations ranging from 2 to 8mmol/L (p<0.05), which was to say that NaF inhibited THP-1 cells proliferation in a dose-dependent manner and this was consistent with the literature [21,22]. In addition, this study also found that with increasing exposure time, the inhibition rates increased when the concentration was fixed (p<0.05), which demonstrates that NaF inhibited THP-1 cells proliferation in a time-dependent manner. But there was no difference between 48 and

72 hrs groups at high concentrations (8mmol/L) (p=0.138), The reason may be that excessive NaF concentration leads to the death of almost all cells [23-25].

The inhibition rate detected by MTT was a general representative of cell death, but it does not distinguish between apoptosis and necrosis. Apoptosis is a genetically controlled active programmed cell death. It is different from necrosis and the unique biological process of apoptosis can significantly reduce the tissue damage. Apoptosis and necrosis have different morphological, biochemical and molecular biological characteristics. The integrity of the cell membrane is one difference between them. Flow cytometry with Annexin V/PI double-staining is based on this principle to detect cell apoptosis: Annexin V can specifically combine with phosphatidylserine (PS), and PI enters cells through the damaged membrane and colors them. In our study, Annexin V/PI double-staining was used to detect the apoptosis rate of THP-1 cells which were treated with various concentration of NaF. The apoptosis rates were 11.53±0.97%, 28±1.78%, 16.11±2.46%, 31.56±3.09%, 42.37±3.51%, 69.52±7.06%. The apoptosis rate had no significant difference (p=0.778 or 0.097) at concentrations of 0.5 mmol/L and 1 mmol/L, which implied that low concentrations (0-1mmol/L) of NaF had no significant effect. The apoptosis rate increased with increasing NaF concentrations, which suggested that NaF induced THP-1 cells apoptosis in a dose-dependent manner at concentrations ranging from 2 to 8 mmol/L, which conformed to some previous studies [13].

In the process of apoptosis, Bcl-2 family is an important regulator of apoptotic pathways. The anti-apoptotic gene Bcl-2 and pro-apoptotic gene Bax were the most important apoptosis-related genes of Bcl-2 family, both of which play an important role in tumor cell apoptosis [17]. Bcl-2 is a major anti-apoptotic gene in Bcl-2 family, which is mainly expressed on the cell membranes (e.g., the outer mitochondrial membrane). Bax is the hottest pro-apoptotic genes in the study of Bcl-2 family, which is mainly located in the cytoplasm. The process of apoptosis regulated by Bcl-2 and Bax is as follows: The death signals stimulate the cells, then induce Bax protein structure change and shift, and bind with Bcl-2 protein of organelle membranes and inhibit its anti-apoptotic effects, eventually destroy cells function, release pro-apoptotic factors, and induce apoptosis [26-30]. Bcl-2 and Bax co-regulate apoptosis. The apoptotic signal weakened when Bcl-2 dominates. On the contrary, the apoptotic signal increases when Bax play a major role. Apoptosis depends on the ratio of Bcl-2 and Bax. In this study, we detected Bcl-2 and Bax levels in THP-1 cells treated with NaF by RT-PCR and found that the Bax and Bcl-2 mRNAs were unchanged after 0-1 mmol/L NaF treatment (p>0.05), which was in accordance to MTT and flow cytometry; meanwhile with NaF concentrations increased, Bax mRNA gradually increased, and Bcl-2 mRNA decreased at concentrations ranging from 2 to 8mmol/L, which demonstrated that NaF upregulated Bax mRNA and reduced Bax mRNA in an dose-dependent manner. Our investigation suggested that apoptosis was induced in THP-1 cells by a decrease in Bcl-2 levels and increase in Bax levels.

In summary, NaF revealed no significant cytotoxicity at lower dose (<1mmol/L). However, at relatively high doses (2-8 mmol/L), NaF induced apoptosis in human acute monocytic leukemia THP-1 cells. Our research revealed that apoptosis was induced in THP-1 cells by a decrease in Bcl-2 levels and increase in Bax levels. In addition, it was also demonstrated that the apoptosis induced by NaF in THP-1 cells was accompanied by a decline in the activation of α-NAE, defining that NaF specifically inhibits the activation of α-NAE which may play a certain role in the induction of apoptosis. However, the specific regulatory mechanism(s) need further investigation.

Conclusions

NaF represses THP-1 cells proliferation and induce THP-1 cells apoptosis in a concentration-dependent manner at concentrations of 2-8mmol/L by down-regulation of Bcl-2 and up-regulation of Bax. The apoptosis induced by NaF in THP-1 cells was accompanied by a decline in the activation of α-NAE, clarifying that NaF, inhibiting the activation of α-NAE, may have played a certain role in the apoptosis. Our current study will be conducive to promoting development of therapy in acute monocytic leukemia.

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Conflict of interests

The authors declare no conflict of interests.

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