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ALTERED FISSION 1 AND MITOFUSIN 1 IN MITOCHONDRIA INDUCED BY EXPOSURE TO FLUORIDE CAUSES APOPTOSIS IN SH-SY5Y CELLS

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ABSTRACT: Chronic fluorosis alters the dynamic balance between mitochondrial fusion and division. The present study investigated the effect of mitochondrial fusion function on apoptosis before or after downregulation of the expression of fission 1 (Fis1) protein in SH-SY5Y cells exposed to excessive fluoride. The expressions of Fis1 and mitofusin 1 (Mfn1) at mRNA and protein levels and the mitochondrial membrane potential level (MMPL) were detected by real-time PCR, Western blot and fluorescence microscopy, respectively. The degree of apoptosis was determined by proliferation toxicity assay. The results showed the upregulation of Fis1 protein and the downregulation of Mfn1 protein, and the decrease in MMPL content due to apoptosis in SH-SY5Y cells exposed to fluoride. Interestingly, these effects were attenuated after the expression of Fis1 protein was inhibited. Taken together, these findings suggested that the toxic effect of fluoride on the target cells might be involved in the Fis1 and Mfn1 protein-related disorder, which eventually led to mitochondrial apoptosis in neurons.

Keywords: Chronic fluorosis; Mfn1 and Fis1 pathogeny; Mitochondria; SH-SY5Y cells.

INTRODUCTION

An excessive intake of fluoride leads to chronic fluorosis, which can cause severe damage to bone, teeth, and other soft organs as well as the central nervous system (CNS).[1] In previous studies, we demonstrated that exposure to fluoride for a prolonged duration elevated the level of free radicals and inhibited the antioxidant defenses, which might constitute the mechanism underlying chronic fluorosis.[2,3] However, the pathogenesis of the disease has not yet been determined.

Reportedly, exposure to fluoride causes detrimental effects on the nervous system, resulting in the apoptosis of neurons.[4] Mitochondria play essential roles in several critical cellular processes, such as the apoptosis caused by fluoride. In neurons, these are vital membrane organelles that are vulnerable to oxidative stress.[5] The decrease in mitochondrial membrane potential is the early stage of injury and apoptosis to the mitochondrial membrane.[6] However, the exact mechanism of mitochondria-derived apoptosis triggered by fluoride necessitates further investigation.

In the present study, we investigated the expression levels of fission-1 (Fis1) and mitofusin-1 (Mfn1) proteins that maintain the structure and function of the mitochondria as well as their correlation with apoptosis due to fluoride exposure.

MATERIALS AND METHODS

Reagents and chemicals: NaF (Sigma, USA), Mitochondrial Membrane Potential Detection Kit (JC-1) (Beyotime Biotechnology, China), anti-Fis1 (PA5-22142), anti-Mfn1 (PA5-38042), goat anti-rabbit IgG (QG221919) (Thermo Fisher Scientific,

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USA), and anti-β-actin (P30002) (Abmart, USA) were purchased. The siRNAs of Fis1 were synthesized by Shanghai GenePharma Co., Ltd, China.

Cell culture and fluoride treatments: SH-SY5Y, a human neuroblastoma cell line (German Collection of Microorganisms and Cell Cultures, Germany) is commonly used to study neurotoxicology. The cells were cultured at 37ºC in 5% (v/v) CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS). After about 60–70% confluency, the cells were exposed to different concentrations of fluoride (0, 0.4, 2, or 4 mmol/L prepared in NaF) for 6, 12, 24, or 48 hr, respectively.

Viability (apoptosis) of cells: Proliferation toxicity was assessed using the CCK-8 kit, which evaluated the viability (apoptosis) of SH-SY5Y cells. The cells were cultured as above and exposed to different concentrations of fluoride (0, 0.05–5 mmol/L for the various time points. An equivalent of 10 μL CCK-8 reagent was added to each well, the OD_{450} measured after 2 hr, and the cell survival rates calculated.

Immunocytochemical staining: The expression and intracellular localization of Fis1 and Mfn1 proteins were detected by immunocytochemistry. Briefly, anti-Mfn1 (1:1000) and anti-Fis1 (1:800) antibodies were applied to the cells on the slides overnight, followed by washing 3 times with PBS, and incubation with goat anti-rabbit IgG (1:5000) for 30 min at room temperature.

Transfection of siRNA and transient cells: To confirm that Fis1 is involved in fluorine-caused neurotoxicity of the cell, small interfering (si)RNA sequences to silence the expression of *Fis1* gene expression in SH-SY5Y cells were synthesized by Shanghai GenePharma Co., Ltd. as follows: siRNAs for Fis1 (human: 5'-GCAAGUACAAUGAUGACAUTT-3', 5'-AUGUCAUCAUUGUACUUGCTT-3'). Scrambled siRNA sequences (5'-UUCUCCGAACGUGUCACGUTT-3', 5'-ACGUGACACGUUCGGAGAATT-3') were used as controls. The lyophilized oligomer was solubilized in DEPC water at a concentration of 20 μM. The cells were seeded at a density of 4×10^5 cells/well in a 6-well plate. Subsequently, 2 mmol/L of sodium fluoride was added for 24 hr. The cells were transfected with the siRNA at 70% confluency using Lipofectamine 3000 Reagent (Invitrogen, Carlsbad, CA, USA) and Opti-MEM medium (Gibco BRL, Paisley, UK) according to the manufacturer's protocol. The *in vitro* transfection of siRNA into SH-SY5Y cells was assessed by NaF exposure after 24 hr, and the mRNA and protein levels of Fis1 were determined.

Real-time fluorescent quantitative PCR: Redzol reagent (SBS Genetech Co., Ltd, Beijing, China) was used to extract the total RNA from SH-SY5Y cells according to the manufacturer's instructions and quantified using Multiskan Spectrum. The purity of RNA was determined by agarose gel electrophoresis. cDNA was synthesized from 5 L of total RNA using the First-strand cDNA Synthesis Kit. Subsequently, the relative expression of *Fis1* and *Mfn1* mRNAs in SH-SY5Y cells was detected by real-time fluorescent quantitative PCR assay. The primers were synthesized by Invitrogen, and *β-actin* was used as an internal reference. The primer sequences were as follows: *β-actin* (131 bp): forward: GCCGACAGGATGCAGAAGG, reverse: TGGAAGGTGGACAGCGAGG; *Fis1* (82 bp): forward: GATGACATCCGTAAAGGCATCG, reverse: AGAAGACGTAATCCCGCTGTT;

Mfn1 (182 bp): forward: TGGCTAAGAAGGCGATTACTGC, reverse: CCGAGATAGCACCTCACCAATG. The reaction conditions were as follows: 50°C preheating for 2 min, 95°C preheating for 2 min, 40 cycles of 95°C for 15 sec (denaturation), 60°C (Bcl-2) for 60 sec (annealing) and 72°C for 1 min (extension). The PCR amplification was performed on ABI StepOne Plus real-time fluorescent quantitative PCR instrument, and the relative expression analyzed using the SDS2.1 software. The results were represented as ΔΔCt and RQ values of $RQ=2^{-\Delta\Delta Ct}$.

Western blotting analysis: Cells were harvested using ice-cold PBS and homogenized in ice-cold RIPA buffer containing protease inhibitors. The concentration of protein in each sample was measured by Ultramicro Spectrophotometer N2000 and an equivalent amount was resolved on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) that was subsequently transferred to PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% (w/v) non-fat skim milk in TBS buffer with Tween 20 (TBS-T) at room temperature for 2 hr and probed with primary antibodies, anti-Fis1, anti-Mfn1, anti-β-actin (1:2000), overnight at 4°C. Subsequently, the membranes were washed three times with TBST, followed by incubation with horseradish peroxidase-conjugated secondary antibody (1:10,000) for 1 hr at room temperature and developed using chemiluminescence reagents (Millipore, Waltham, MA, USA). The intensities of the immunoreactive bands were quantitated using Image J software.

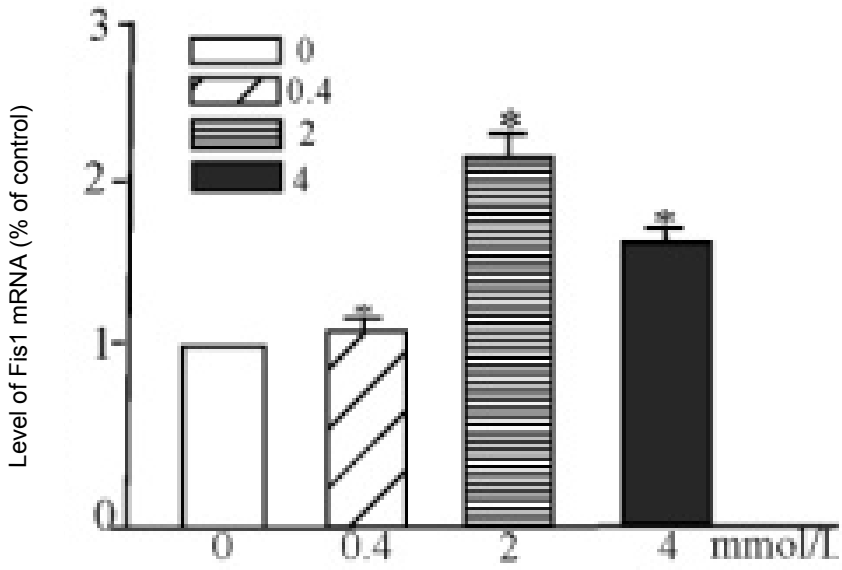
Assay for mitochondrial membrane potential level (MMPL): MMPL was detected by Mitochondrial Membrane Potential Assay Kit (JC-1) according to the manufacturer's protocol. When the membrane potential of the mitochondria was higher, JC-1 was accumulated in the matrix of the mitochondria that emitted red fluorescence (normal). On the other hand, JC-1 alone could not be clustered in the matrix of the mitochondria, and the JC-1 monomer emitted green fluorescence (abnormal) that was detected by fluorescence microscope and analyzed using Image J software. The ratio of the optical density of synthetic fluorescence was estimated as the expression of *MMPL*.

Statistical analysis: Data are expressed as the mean ± standard deviation (SD) of at least three independent replicates. Statistical analysis was performed by parametric or nonparametric test using SPSS version 19 (IBM, New York, USA). The parametric test includes one-way ANOVA or independent samples t-test, and the post-hoc test was performed by the LSD method. $P<0.05$ was considered as statistically significant.

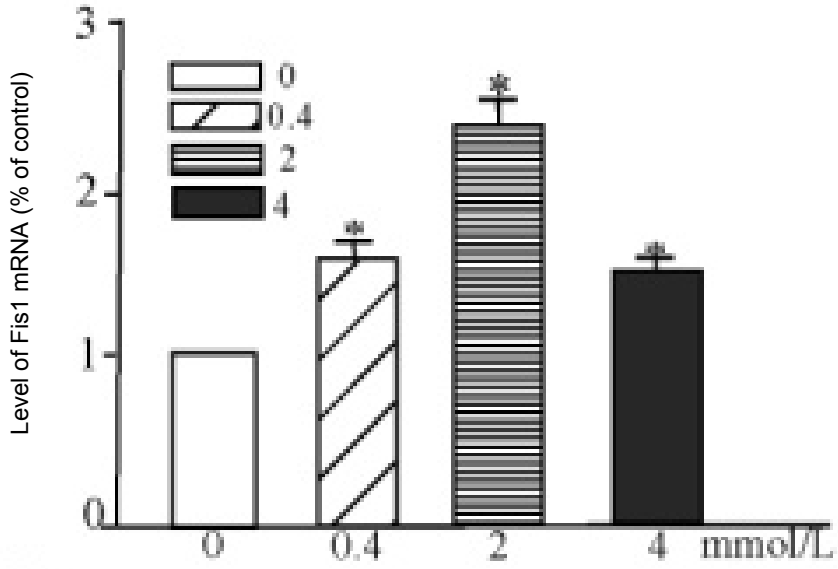
RESULTS

Viability (apoptosis) of SH-SY5Y cells exposed to fluoride: The SH-SY5Y cells were treated with different concentrations of F^- (prepared with NaF) for 6, 12, 24, and 48 hr, and the proliferation of the cells was found to be inhibited in a dose- or time-dependent manner. Consequently, three concentrations (0.4, 2, and 4 mmol/L) of F^- were selected for fluoride exposures to the cultured cells.

Expression of Fis1 and Mfn1 mRNA: In comparison with the untreated controls, the expression level of *Fis1* mRNA was increased after fluoride exposure in SH-SY5Y cells in a significant dose- or time-dependent manner (Figures 1A–1D), while that of *Mfn1* mRNA was reduced (Figures 2A–2D).

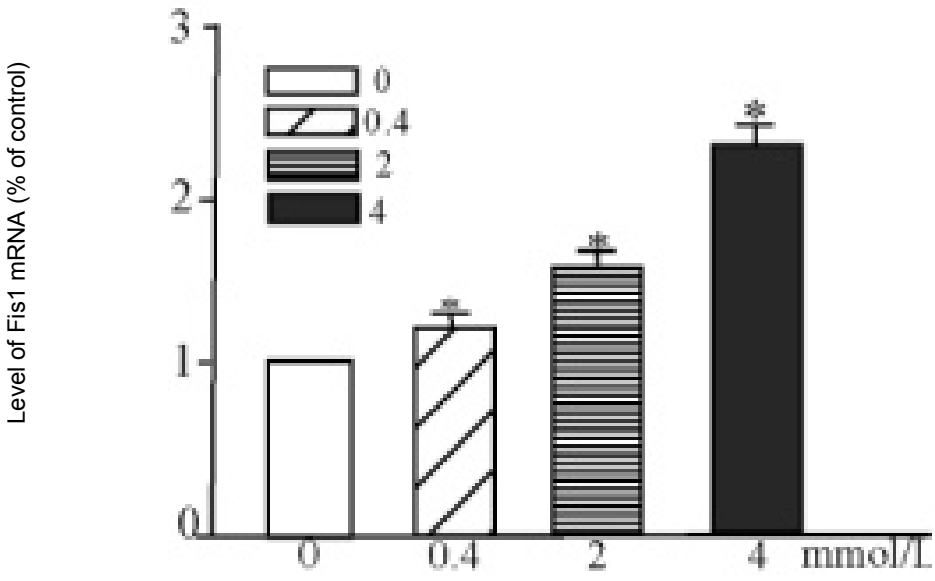


1A

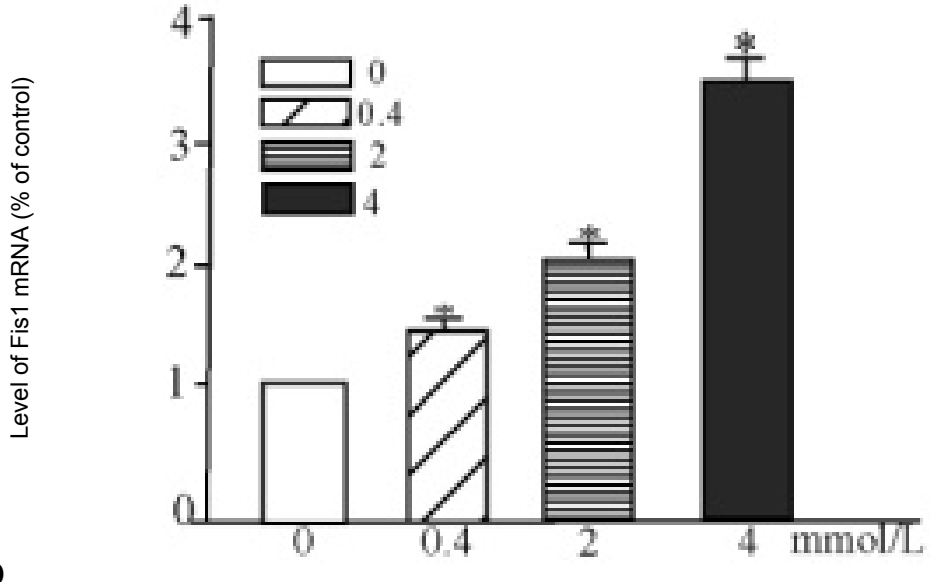


1B

Figures 1A–1D. Fluorine exposure upregulates Fis1 mRNA expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for different time periods of 6 hr (1A), 12 hr (1B), 24 hr (1C), and 48 hr (1D). Real-time PCR and quantitative analyses determined the Fis1 mRNA expression. *P<0.05 vs. vehicle-treated cells (n=3/group).

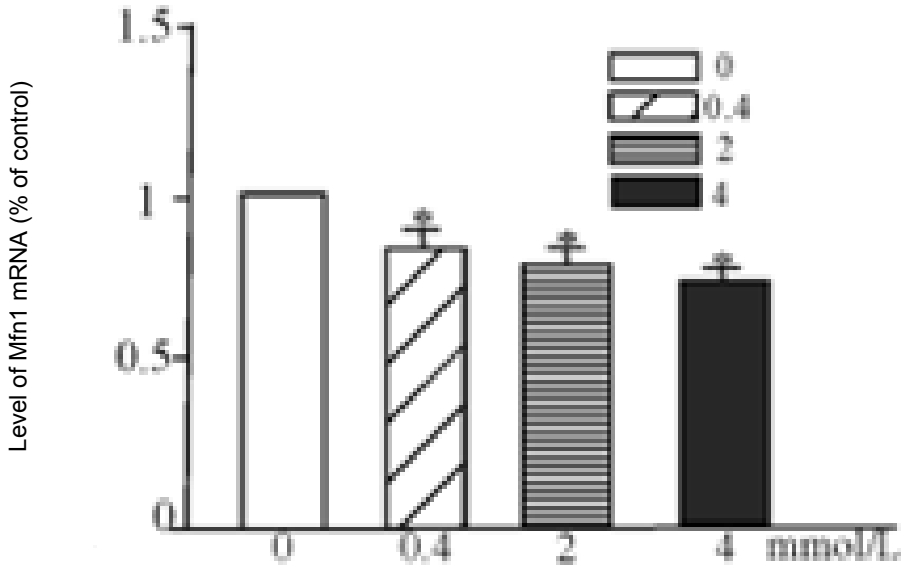


1C

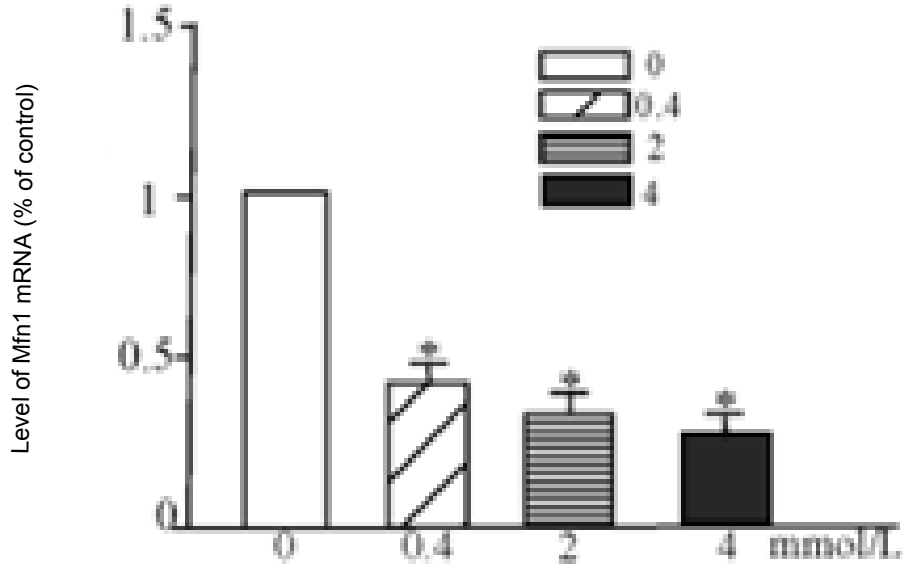


1D

Figures 1A–1D. Fluorine exposure upregulates Fis1 mRNA expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for different time periods of 6 hr (1A), 12 hr (1B), 24 hr (1C), and 48 hr (1D). Real-time PCR and quantitative analyses determined the Fis1 mRNA expression. *P<0.05 vs. vehicle-treated cells (n=3/group).

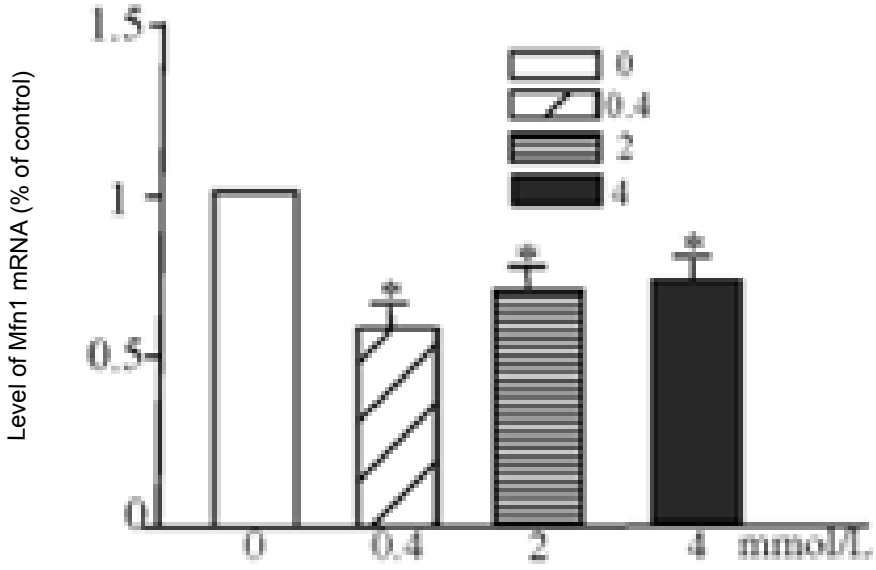


2A



2B

Figures 2A–2D. Fluorine exposure downregulates Mfn1 mRNA expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for the different time periods of 6 hr (2A), 12 hr (2B), 24 hr (2C), and 48 hr (2D). Real-time PCR and quantitative analyses determined the Mfn1 mRNA expression. *P<0.05 vs. vehicle-treated cells (n=3/group).



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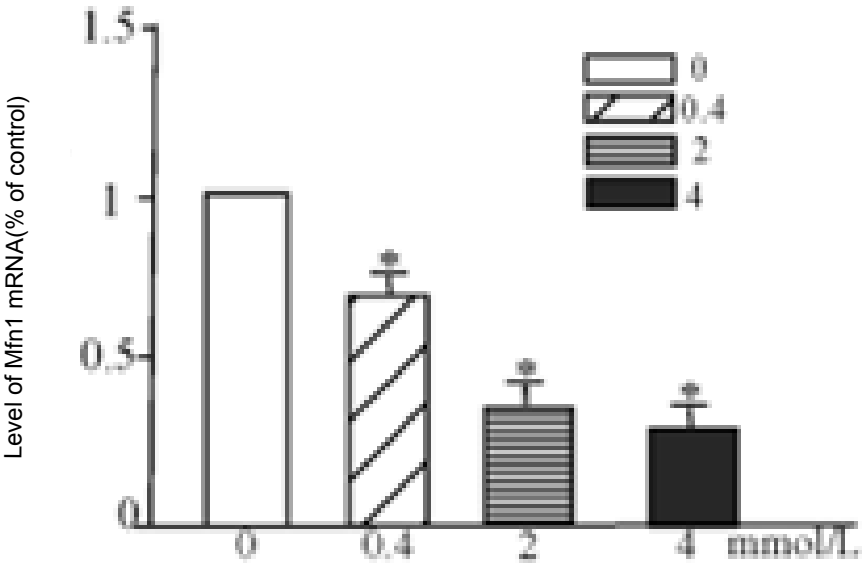
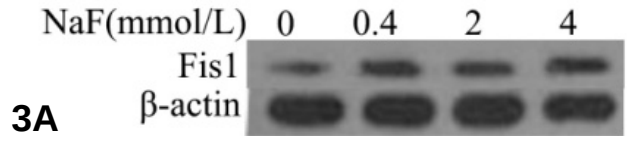


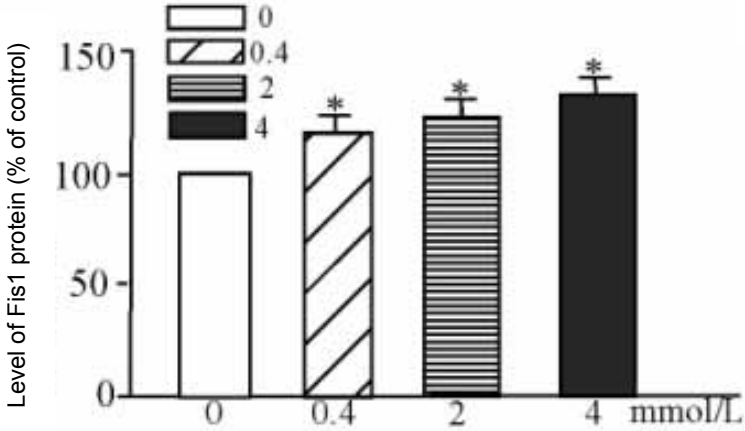
2D

Figures 2A–2D. Fluorine exposure downregulates Mfn1 mRNA expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for the different time periods of 6 hr (2A), 12 hr (2B), 24 hr (2C), and 48 hr (2D). Real-time PCR and quantitative analyses determined the Mfn1 mRNA expression. *P<0.05 vs. vehicle-treated cells (n=3/group).

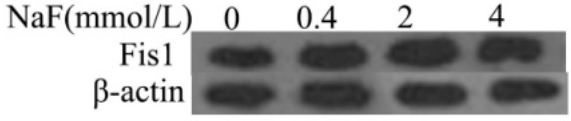
Levels of Fis1 and Mfn1 proteins: In comparison with the untreated controls, the expression level of Fis1 protein in SH-SY5Y cells treated with different concentrations of F^- was increased (Figures 3A–3H), while that of Mfn1 reduced (Figures 4A–4H) in a dose- or time-dependent manner.



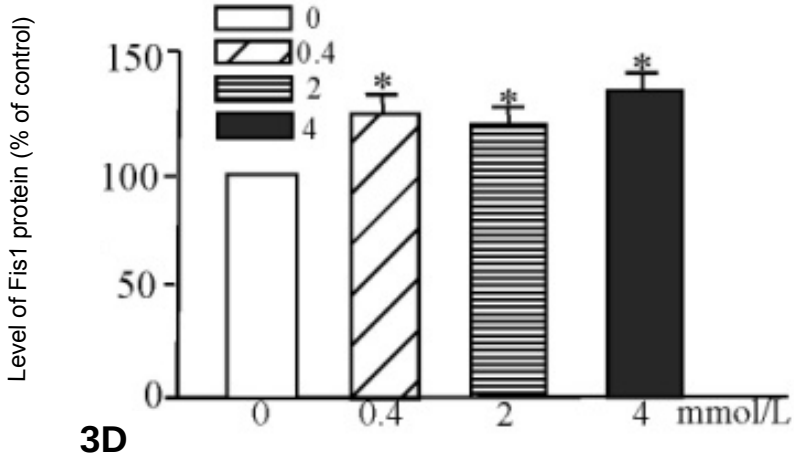
3A



3B

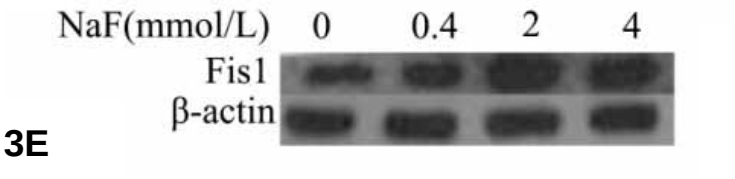


3C

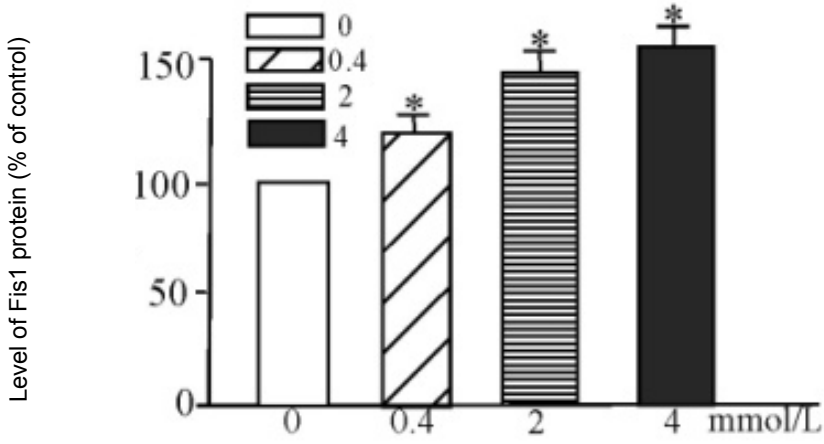


3D

Figures 3A–3H. Fluorine exposure upregulates Fis1 protein expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for different time periods of 6 hr (3A and 3B), 12 hr (3C and 3D), 24 hr (3E and 3F), and 48 hr (3G and 3H). Western blot and quantitative analyses determined the Fis1 protein expression. β-Actin was used as a loading control. *P<0.05 vs. vehicle-treated cells (n=3/group).



3E



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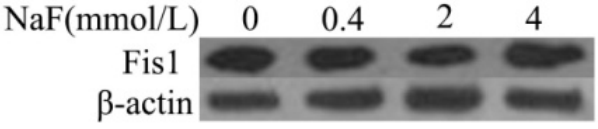
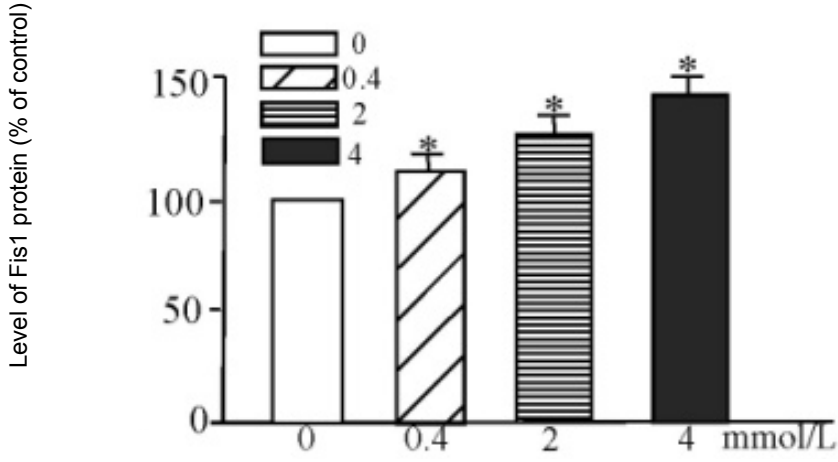
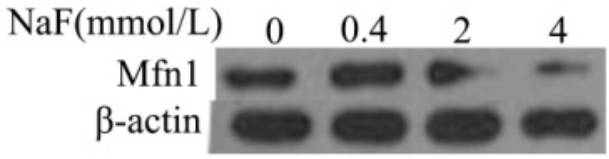


3G

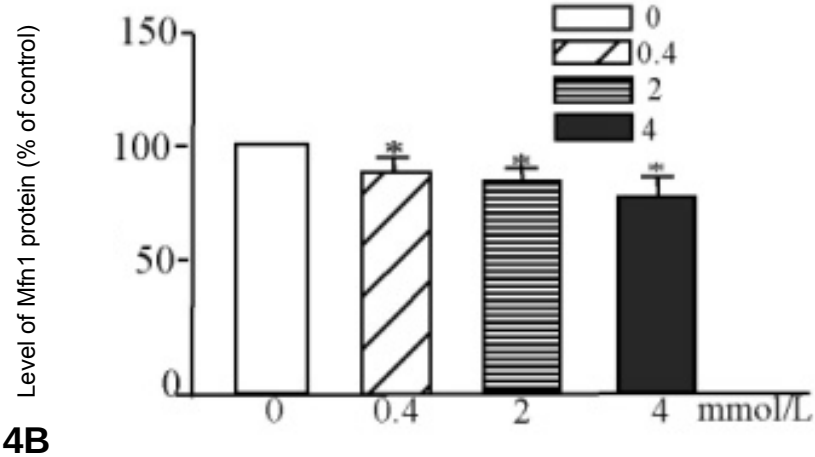


3H

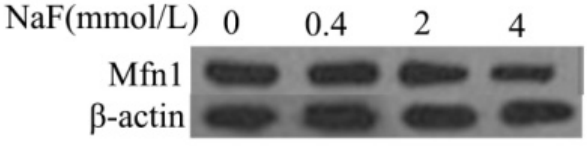
Figures 3A–3H. Fluorine exposure upregulates Fis1 protein expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for different time periods of 6 hr (3A and 3B), 12 hr (3C and 3D), 24 hr (3E and 3F), and 48 hr (3G and 3H). Western blot and quantitative analyses determined the Fis1 protein expression. β-Actin was used as a loading control. *P<0.05 vs. vehicle-treated cells (n=3/group).



4A



4B



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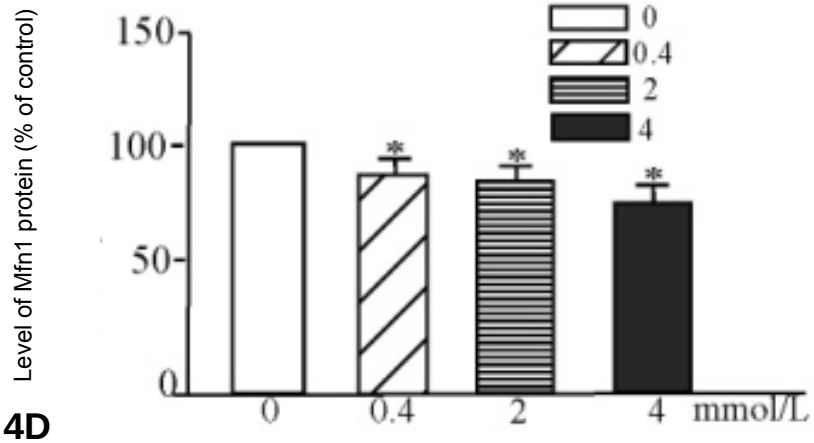
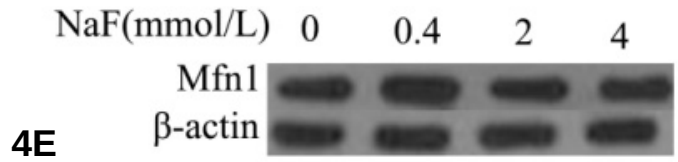


4D

Figures 4A–4H. Fluorine exposure downregulates Mfn1 protein expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for different time periods of 6 hr (4A and 4B), 12 hr (4C and 4D), 24 hr (4E and 4F), and 48 hr (4G and 4H). Western blot and quantitative analyses determined the Mfn1 protein expression. β-Actin was used as a loading control. *P<0.05 vs. vehicle-treated cells (n=3/group).



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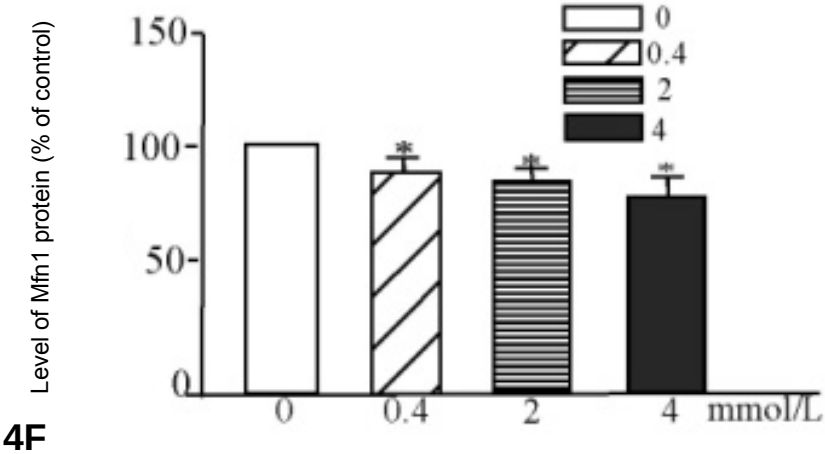

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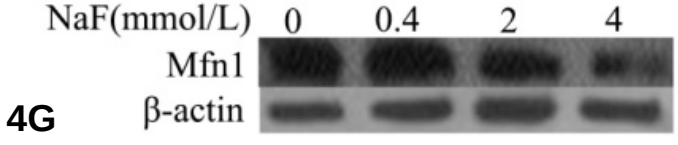
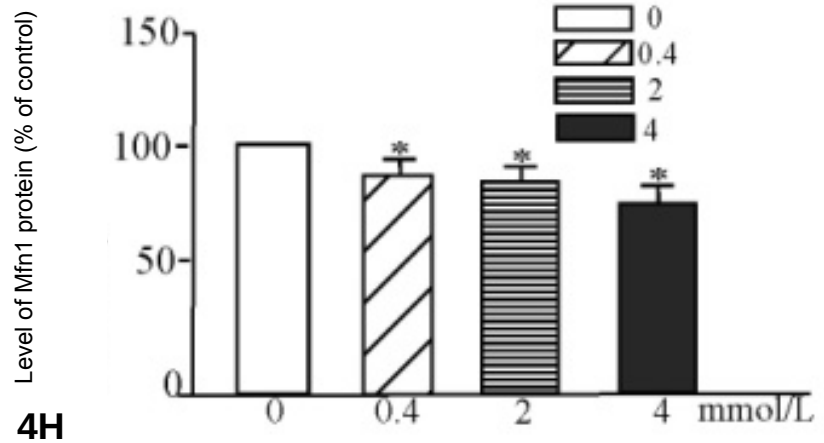


4G



4H

Figures 4A–4H. Fluorine exposure downregulates Mfn1 protein expression in a concentration- and time-dependent manner in SH-SY5Y cells. SH-SY5Y cells were exposed to various concentrations of NaF (0, 0.4, 2, and 4 mmol/L) for different time periods of 6 hr (4A and 4B), 12 hr (4C and 4D), 24 hr (4E and 4F), and 48 hr (4G and 4H). Western blot and quantitative analyses determined the Mfn1 protein expression. β-Actin was used as a loading control. *P<0.05 vs. vehicle-treated cells (n=3/group).

Effects of the fluoride on the MMPL: Compared to the control cells (red fluorescence), MMPL in the fluoride exposure groups was significantly decreased. The green fluorescence (abnormal MMPL) in the high-fluoride group was brighter than the control or the low-fluoride group (Figure 5 and the Table).

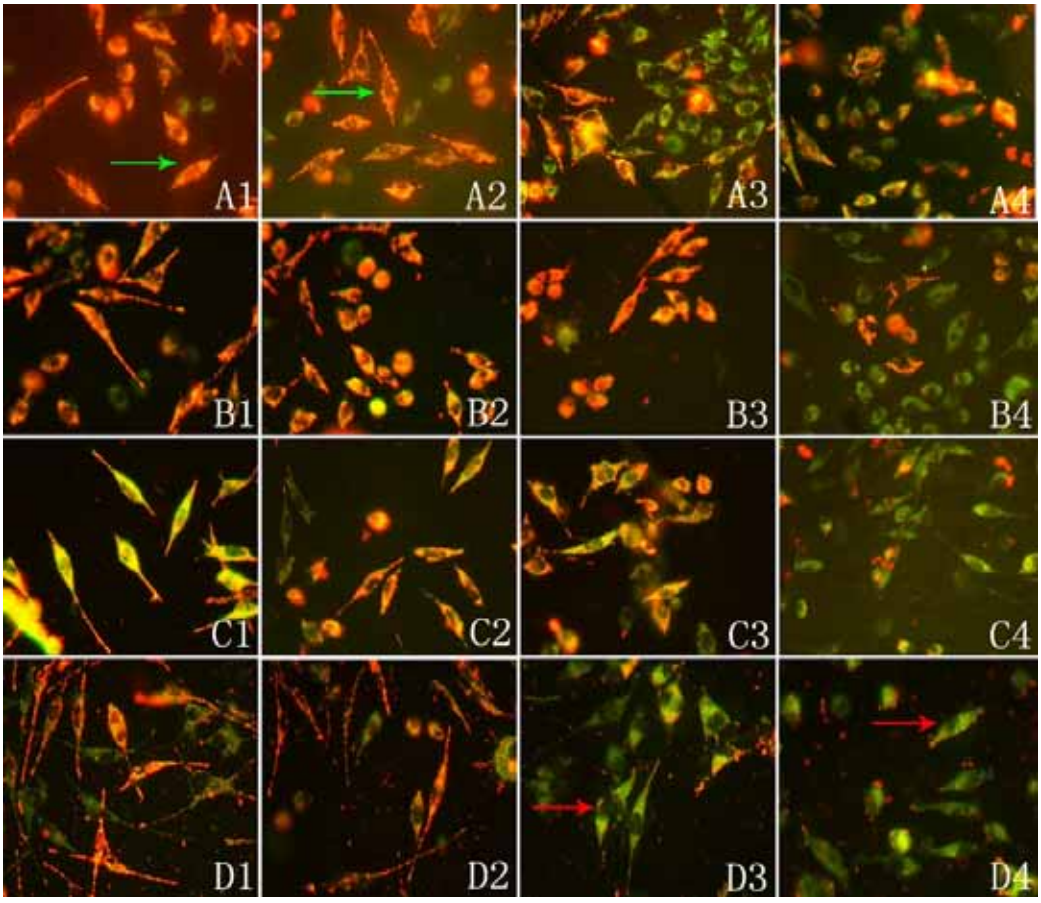


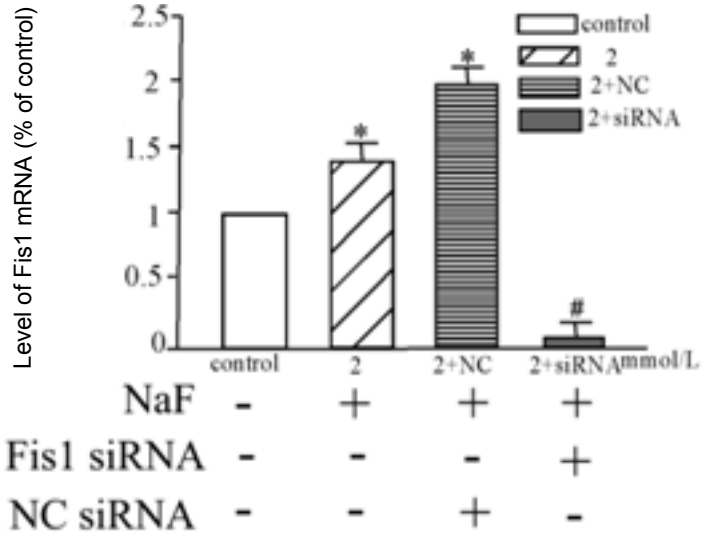
Figure 5. The red and green fluorescence of the MMPLs of SH-SY5Y cells: SH-SY5Y cells were exposed to various concentration of NaF (0, 0.4, 2, and 4 mmol/L) for different time periods of 6 hr (A1, A2, A3, and A4), 12 hr (B1, B2, B3, and B4), 24 hr (C1, C2, C3, and C4), and 48 hr (D1, D2, D3, and D4). The green arrow indicates the normal MMPLs (red cells), and the red arrow indicates the decrease in MMPLs (green cells).

Table. Effect of fluoride on mitochondrial membrane potential of SH-SY5Y cells

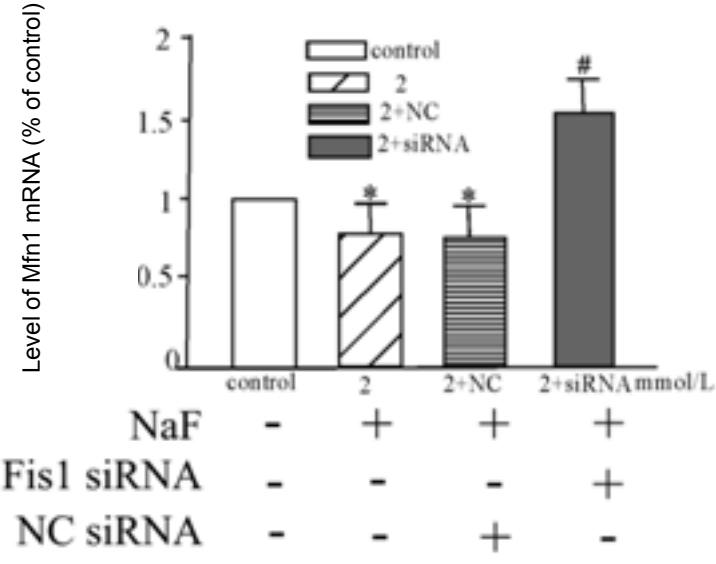
Group NaF (mmol/L)	Time (hr)			
	6	12	24	48
0	1.63±0.17	1.12±0.14	0.82±0.01	1.29±0.02
0.4	1.03±0.05*	0.94±0.06	0.71±0.08	1.11±0.01*
2	1.01±0.09*	0.75±0.13*	0.67±0.12*	0.82±0.01*
4	0.79±0.04*	0.62±0.09*	0.59±0.10	0.56±0.04*

*P<0.05 vs. vehicle-treated cells. Data were analyzed using one-way ANOVA, followed by LSD post-hoc analysis. Data are expressed as means±SD (n=3/group).

Effects of Fis1 gene silencing on the expressions of Fis1 and Mfn1 at mRNA and protein levels: The specific siRNA for *Fis1* gene silencing effectively attenuated the elevated level of *Fis1* mRNA and the decreased level of *Mfn1* mRNA induced by exposure to fluoride (Figure 6).



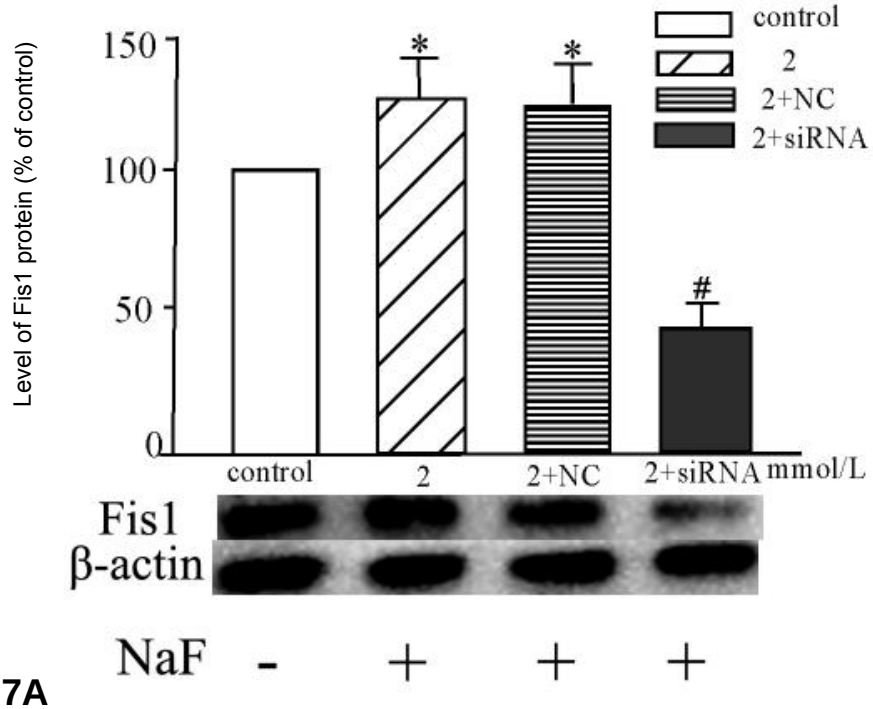
6A



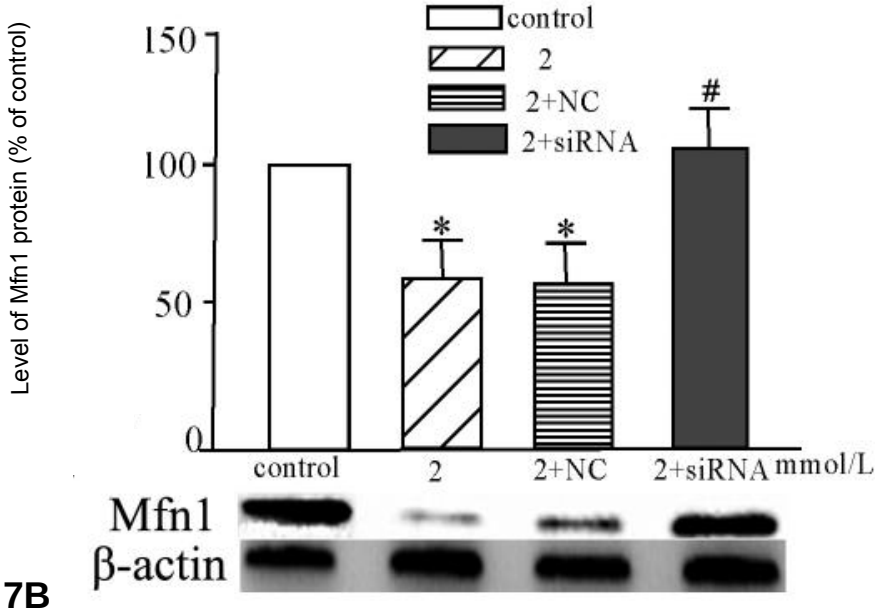
6B

Figure 6. Fis1 siRNA alters Fis1 or Mfn1 mRNA levels. Cells were exposed to blank control group (control), fluoride group (with 2 mmol/L fluoride ion, 2), fluoride negative control group (with 2 mmol/L fluoride + non-specific siRNA, 2 + NC), and the gene-silencing group (with 2 mmol/L fluoride ion + specific siRNA-Fis1, 2 + siRNA). Quantitative analysis was performed to evaluate the expression of Fis1 mRNA (6A) and the expression of Mfn1 mRNA (6B). At least three independent experiments were analyzed. *P<0.05 compared to the control cells, #P<0.05 compared to the fluoride group cells.

Moreover, the specific siRNA effectively attenuated the expression of both proteins in the cells exposed to fluoride, which was consistent with that of the corresponding mRNA (Figure 7).



7A



7B

Figures 7A and 7B. Fis1 siRNA alters Fis1 or Mfn1 protein levels. Cells were exposed to blank control group (control), fluoride group (with 2 mmol/L fluoride ion, 2), fluoride negative control group (with 2 mmol/L fluoride + non-specific siRNA, 2 + NC), and the gene-silencing group (with 2 mmol/L fluoride ion + specific siRNA-Fis1, 2 + siRNA). Western blot analyses were performed to evaluate the expression of Fis1 protein (7A), the expression of Mfn1 protein (7B). At least three independent experiments were analyzed. *P<0.05 compared to the control cells, #P<0.05 compared to the fluoride group cells.

Effects of Fis1 gene silencing on MMPL: The level of the MMPL in the cells exposed to fluoride (2 mmol/L F^-) was reduced by 24% as compared to the controls, while silencing the *Fis1* gene attenuated the abnormal MMPL such that it was similar to the baseline (Figure 8).

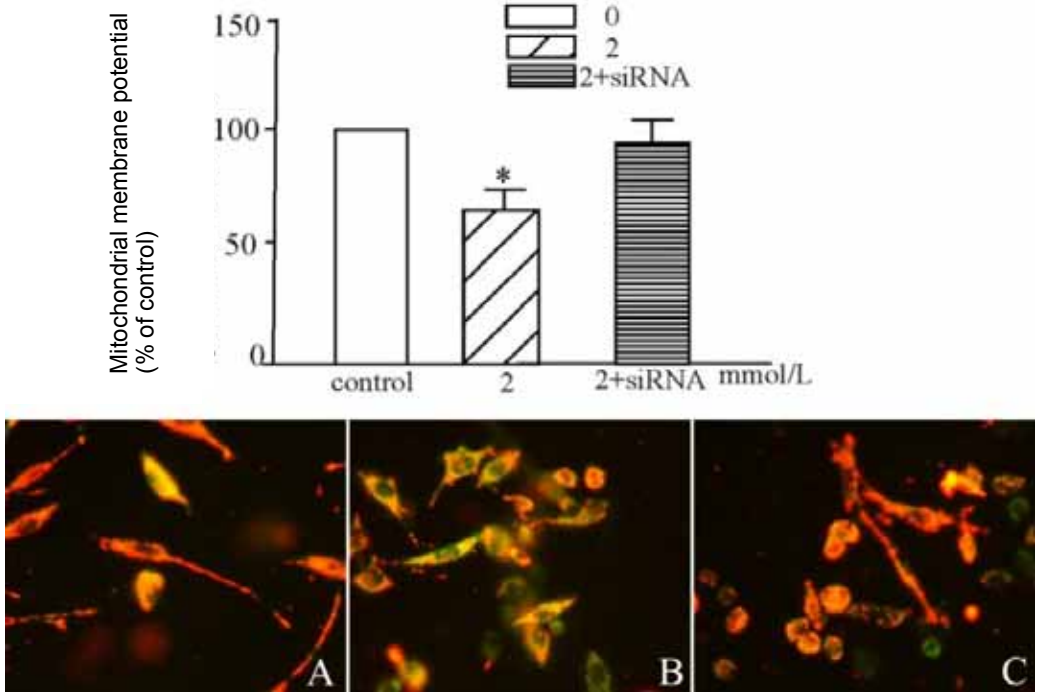


Figure 8. The changes of red and green fluorescence of the MMPLs of SH-SY5Y cells after Fis1 silencing; A: control group (control), B: fluoride group (2 mmol/L sodium fluoride, 2), C: Fis1 silencing group (2 mmol/L sodium fluoride + siRNA interference, 2 + siRNA). At least three independent experiments were analyzed. *P<0.05 compared to the control cells.

DISCUSSION

Chronic fluorosis causes severe injury to multiple organs and systems.[7-8] Interestingly, excessive fluoride can lead to profound changes in the CNS in patients or experimental animals with chronic fluorosis, resulting in a decline in intelligence.[9] However, the mechanism underlying the pathogenesis of this disease in the CNS is yet to be elucidated.

In a previous study, we found altered morphology of mitochondria and the dynamic balance of mitochondrial proteins (Fis1, Drp1, and Mfn1) in the neurons of rat brains in animals with chronic fluorosis.[10] A changed distribution of mitochondria in the cell body and synapse and damaged mitochondrial DNA in the neurons were observed, indicating the mitochondrial dysfunction may be related to the brain damage caused by fluoride.[10]

In chronically poisoned human brains, brain F^- concentration is approximately 2–10 ppm (0.11–0.53 mM). [11] According to the current experiments, the rate of proliferation of SH-SY5Y cells was >90% with 4 mM. Thus, we speculated that these tumor cells are characterized by a high proliferation rate and regenerating

ability that imparts resistance to the damage caused by an adverse external environment. For example, these tumor cells are more resistant and tolerant to fluoride toxicity than brain cells *in vivo*. Yj et al. also confirmed that the apoptotic rate of animal brain cells *in vivo,* with F^- in the range of 5–50 ppm, is about 3–10-fold that of the controls, while only 1.2–1.4-fold in SH-SY5Y cells *in vitro*.[7] Therefore, the *in vivo* or *vitro* concentration of fluoride toxicity should be selected according to individual cell differences, and not completely on the *in vivo* conditions. Consequently, in the current study, the concentrations of F^- used were 0.4, 2, and 4 mM fluoride, which were higher than the F^-concentration in chronically poisoned human brains.

Owing to the mitochondrial dysfunction of the rat brain mentioned above, we focused on the mitochondrial mechanism using the SH-SY5Y cell model *in vitr*o. Typically, mitochondria rely on the mitochondrial membrane potential (MMP)-generated ATP for the physiological function of cells.[12-13] In the present study, we found that the MMPL in SH-SY5Y cells was decreased by the treatment with fluoride, and that the decrease was significantly correlated to the dose and time of the exposure to the fluoride ions. When the level of MMP decreases, the cells exhibit a decline in cell viability and growth, followed by apoptosis, given the data published in reference.[7] The mitochondrial dysfunction has been shown to participate in the induction of apoptosis and to be the core of the pathway. Furthermore, the changes in the membrane potential might be attributed to the opening of the mitochondrial permeability transition pore (MPTP). These pores allow the passage of ions and small molecules that release the apoptogenic factors and cause the loss of oxidative phosphorylation, which in turn, results in the decoupling of the respiratory chain and the release of cytochrome c into the cytosol as a distinctive feature of the early stages of programmed cell death.[14] In the current study, the mechanism underlying the fluoride-induced loss of MMP in the cells is an early event in the apoptotic process that might be linked to MPTP.

With the opening of the MPTP and the loss of the MMP, it is difficult to maintain the potential difference of the electron transport chain (ETC) needed to produce ATP, and then, the membrane of mitochondria is destabilized to start the membrane splitting chain reactions that alter the morphology of mitochondria.

The membrane splitting chain reactions of mitochondria are based on a dynamic balance between membrane fusion and division. In the physiological state, the balanced state increases the mitochondrial mitosis leading to mitochondrial fragmentation.[15,16] The mitochondrial balance was adjusted by proteins, including Mfn1 and Fis1. The Mfn1 protein reduces the mitochondrial fragmentation and participates in the reduced process of mitochondrial apoptosis to maintain the cell viability.[17,18] Also, the Fis1 protein is a crucial factor that mediates mitochondrial cleavage. Thus, Mfn1 and Fis1 proteins maintain the normal structure and function of mitochondria to reduce cell apoptosis.[19,20]

In the current study, the changes in the protein and mRNA of mitochondrial fusion and fission were observed before or after silencing the expression of *Fis1* gene in SH-SY5Y cells exposed to fluoride. The results showed that the expression of Mfn1 and Fis1 proteins and mRNAs in SH-SY5Y cells was significantly altered at the different time periods *in vitro*. Moreover, the expression of Mfn1 protein and mRNA was

decreased with an increased exposure dose of fluoride for a prolonged period, while that of Fis1 protein and mRNA was increased. In recent years, numerous studies have shown that the division of mitochondria is closely related to apoptosis,[21] and, especially, the abnormal fusion and fission proteins, such as Mfn1 and Fis1. The overexpression of Fis1 causes mitochondrial fragmentation and cell apoptosis.[17,22] In addition, Fis1 is the first receptor identified to recruit DRP1, another fission protein to directly split the membrane and it is speculated to interact with DRP1 via its two tetratricopeptide repeat (TPR)-like motifs.[23] Thus, the current findings suggested that the loosening of MMP on the SH-SY5Y cells induced by fluoride might disrupt the membrane stability of mitochondria, and lead to the recruiting of the Fis1 protein. Subsequently, the Mfn1 protein is downregulated to start the mitochondrial fission, which is involved in the apoptosis of SH-SY5Y cells.

In order to elucidate the mechanism of Fis1 protein in damaged MMPLs, reduced cell proliferation, and fluoride-induced apoptosis, RNAi was used for silencing *Fis1* in SH-SY5Y cells. The results showed that the expression of Fis1 protein and mRNA in the silenced group was significantly lower than that in the fluoride group, while the expression of Mfn1 protein and mRNA was restored to a normal level. Interestingly, the content of MMPLs in the silenced group was significantly higher than that in the fluoride group. In addition, the loss of mitochondrial membrane potential is an early apoptotic signal.[14] Consequently, the mitochondrial mitosis is initiated to adapt to an altered cellular environment with lower MMP, which might be conducive for lysosomes to swallow the smaller fragments in the mitochondria.[24] In this study, the low expression of Fis1 in SH-SY5Y cells was effected by RNAi, while that of MMPLs was resumed even if the cell was attacked by the fluoride ion. Thus, even if apoptosis factors are present, the low level of mitochondrial fission might interfere in loosening of mitochondrial MMP that would be returned to the normal state for reduced apoptosis. The feedback mechanism between MMP and mitochondrial fission are vital for cell survival following the assault by the fluoride ion.

In the present study, the mitochondrial damage, resulting from either fusion or fission, in apoptosis caused by fluoride was investigated. Recently, an increasing number of pathways of mitochondrial damage induced by fluoride have been mentioned, i.e., p53 pathway or mitochondrial autophagy, but the protective effect exerted by changing the Fis1 or p53 pathway could alleviate the apoptosis of nerve cells and neurotoxicity from fluoride.[25] Thus, we speculate that apoptosis from the mitochondrial damage caused by fluoride involves multiple pathways.

CONCLUSIONS

In conclusion, the loss of MMP in SH-SY5Y cells exposed to fluoride *in vitro* led to membrane splitting, which in turn, altered the morphology of mitochondria due to the abnormal expression of Mfn1 and Fis1 proteins. The effects were alleviated by silencing the expression of *Fis1* and this might be involved in fluoride-related apoptosis in SH-SY5Y cells. These results suggest that morphological and functional changes in mitochondria might play a critical role in the pathogenesis of the damage to cells caused by fluoride.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study.

ETHICAL APPROVAL:

No procedures were performed involving human participants.

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