Exogenous transforming growth factor-β1 prevents the inflow of fluoride to ameleoblasts through regulation of voltage-gated chloride channels 5 and 7

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Abstract. Dental fluorosis is a global issue. Although there are multiple causes of dental fluorosis, the precise mechanism remains controversial. Previous studies have demonstrated that extracellular fluoride may promote an accumulation of fluoride ions in ameloblasts, which may induce oxidative and endoplasmic reticulum stresses, leading to dental fluorosis. However, the exact process by which fluoride ions enter cells has not been determined. In the present study, intracellular fluoride concentration was determined using a newly developed specific fluorescent probe called probe 1. Under high extracellular fluoride concentrations, the fluorescence intensity of the ameloblasts increased, however, exogenous transforming growth factor-β1 (TGF-β1) was able to inhibit the increase. Furthermore, changes in the expression of the voltage-gated chloride channels 5 and 7 (ClC5 and ClC-7), which are responsible for the transport of fluoride were investigated. The results indicated that fluoride reduced the expression of endogenous TGF-β1 and increased the expression of ClC-5 and ClC-7. Additionally, exogenous TGF-β1 reduced the expression of ClC-5 and ClC-7. The results of the present study indicate that exogenous TGF-β1 may prevent accumulation of fluoride in ameloblasts through the regulation of ClC-5 and ClC-7 under high extracellular fluoride concentrations.

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Abbreviations: ClC-5, voltage-gated chloride channel 5; ClC-7, voltage-gated chloride channel 7; CICs, voltage-gated chloride channels; CFTR, cystic fibrosis transmembrane conductance regulator; CLCA, calcium-activated chloride channels; CLICs, chloride intracellular channels; ECM, extracellular matrix; HA, hydroxyapatite; HF, hydrogen fluoride; KLK4, kallikrein 4; LS8, the ameloblast-like cell line; RT-qPCR, reverse transcription-quantitative PCR; TGF-β1, transforming growth factor-β1

Key words: fluoride, chloride channels, transforming growth factor-β1, ameloblasts, probe

Introduction

The supplementation of drinking water with fluoride is an established method to prevent dental caries (1). According to the Dean index, the optimal concentration of fluoride in drinking water is 1 ppm (2). Nevertheless, in In many areas of the world, the natural fluoride levels in drinking water exceed the recommended concentration (1). In some areas, the natural fluoride levels in drinking water are extremely high, such as in Colorado (11.2 mg/l), Oklahoma (12.0 mg/l), New Mexico (13.0 mg/l) and Idaho (15.9 mg/l) (3). With the development of dentistry, fluoride has also been added to dental materials, including toothpaste, varnish, foam and dental resin, to prevent the occurrence of caries. All these fluoride additions increase the morbidity of dental fluorosis (3), which is currently a worldwide problem. A small 'pea-sized' amount of toothpaste would contain 0.36-0.72 mg fluoride, which would increase the risk of dental fluorosis in children (4). In America, the prevalence of dental fluorosis in children aged 15-17 years increased from 22.6% in the 1986-1987 to 40.6% in the 1999-2004, which was provided by the National Health and Nutrition Examination Survey (5). Although there are multiple causes of dental fluorosis, the precise mechanism remains controversial (6,7). The regulation of intracellular and extracellular pH is an important mechanism that may lead to dental fluorosis. According to the acid hypothesis (8), fluoride ions are unable to enter the cell, whereas hydrogen fluoride (HF) can readily cross the cell membrane under acidic conditions (9). An increased fluoride concentration in the cytoplasm may induce oxidative stress by reducing the activity of antioxidant enzymes, thereby affecting a variety of structures and processes of normal cells due to reactive oxygen species accumulation (8,10-12). Furthermore, fluoride in the cytoplasm may induce endoplasmic reticulum (ER) stress, including the phosphorylation of the eukaryotic initiation factor 2 (eIF2), which may result in a decrease in the overall protein production, including secretion of the protease kallikrein 4 (KLK4) (8,13-15). However, studies on fluoride transport have primarily used fibroblasts and epithelial cells from the rat stomach and urinary bladder,

hamster cheek pouch and renal tubules of several species, and very little research has been carried out in ameloblasts (9,16). Sharma *et al* (13) reported that a low pH environment in the maturation stage of ameloblasts facilitated the uptake of fluoride. This hypothesis was tested by measuring changes in stress-related genes and proteins. Fluorescent probes are a more intuitive method to observe the accumulation of fluoride in ameloblasts (17). However, limitations remain in the majority of the reported fluorescent fluoride probes in terms of determination and bioimaging (17-20). For example, pyrene-based dye lies within the ultraviolet-visible light range, increasing the difficulties in targeting fluoride in living animals (17,18). Although the probes with the lipophilic TBDPS moiety have high selectivity, their performance in apparent optical signal changes to the naked-eye remains poor (17,19,20). To improve on these methods, a highly selective ratiometric visual and red-emitting fluorescent dual-channel, probe 1, was developed. The probe contains the hydrophilic compound 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) and the lipophilic tert-butyldiphenylsilyl (TBDPS); therefore, it can enter cells easily (21,22). Additionally, probe 1 possesses a latent internal charge transfer structure, which makes it detect signals to the naked-eye (21,22). Through cleavage of the Si-O bond, fluoride transforms probe 1 into a red-emitting fluorescent form. Therefore, probe 1 reflects the distribution of the fluoride ion may be used to determine fluoride concentration (17,21,22).

The epithelial transport process of fluoride relies on ion channels. To the best of our knowledge, no specialized fluoride channels have been identified. It is generally accepted that fluoride ions pass through the membrane via chloride channels (23). There are differences in the permeability and conductivity of fluoride between different chloride channels (23). Simchowitz (24). found that human neutrophils were selective for haloid ions, and the order of affinity for these ions was $Cl^->Br^->F^->I^-$, which were reverse-transported in a 1:1 ratio to extracellular Cl^-. Studies on the epithelium of the respiratory tract have revealed that its chloride channels are highly selective for anions and that their ionic permeability to halogens is in the order of $I^->Br^-\geq Cl^->F^-$ (25,26). Additionally, Anderson *et al* (27) demonstrated that the order of selectivity of cystic fibrosis transcription factor for anions was $Br^->Cl^->I^->F^-$. In mammals, several classes of chloride channels have been found, including voltage-gated chloride channels (ClCs), the cystic fibrosis transmembrane conductance regulator (CFTR), calcium-activated chloride channels (CLCAs) and chloride intracellular channels (CLICs) (28). There are nine ClCs, namely ClC-1 to ClC-7, ClC-Ka, and ClC-Kb. ClCs-3-7 function as the main Cl^-/H^+-exchangers, and facilitate vesicular acidification by shunting currents of proton pumps and by increasing vesicular Cl^- concentration (29,30). ClCs control the electrical excitability of muscles or neurons, extra- and intracellular ion homeostasis and transepithelial transport (30). Hou *et al* (31) found that ClCs-1-7 were expressed in tooth germs. ClC-5 has been reported in the dental lamina, inner enamel epithelium, stratum intermedium, outer enamel epithelium, odontoblasts and ameloblasts (32). CLCN7 mRNA has been reported in ameloblasts at the maturation-stage (33). CFTR plays an important role in transportation of bicarbonate into the enamel space to buffer protons in ameloblasts and is located on the apical plasma membrane during the maturation stage of ameloblasts (34). Lacruz *et al* (35) reported that the mRNA level of CFTR in rat ameloblasts was higher at the early-mid and mid-late maturation stages than at the secretory stage. Furthermore, previous studies have indicated that CFTR is a critical factor in the regulation of pH during the maturation of ameloblasts and is essential for enamel mineralization (34,36,37). Duan *et al* (38) demonstrated that CFTR inhibition and treatment with CFTR siRNA may increase intracellular pH. In our previous study, ClC-5 and ClC-7, which are Cl^-/H^+-exchangers related to tooth development and pH regulation were the focus (30).

As a member of the transforming growth factor-β (TGF-β) superfamily, TGF-β1 regulates cellular biological processes, such as proliferation, differentiation, apoptosis and extracellular matrix (ECM) protein production (39). Furthermore, it plays an important role in tooth development (32,40). Duan *et al* (32) indicated that ClC-5 regulates dentine development through the TGF-β1 pathway. Previous studies have revealed that fluoride affects enamel protein content via TGF-β1-mediated KLK4 inhibition (41,42). TGF-β1 may also impair the expression and function of chloride ion channels in other epithelial cells, including alveolar, bronchial, vas deferens and nasal polyp epithelial cells (43-46). However, whether the expression and function of chloride ion channels in ameloblasts is regulated by TGF-β1 remains to be elucidated.

In the present study the correlation between TGF-β1 and the intracellular fluoride concentration at a high extracellular fluoride concentration was investigated. Furthermore, the effects of exogenous TGF-β1 on ClC-5 and ClC-7 under a high extracellular fluoride concentration were studied.

Materials and methods

Cell culture. The ameloblast-like cell line (LS8) was a gift from Prof. Malcolm L. Snead (University of Southern California, USA), and was cultured in DMEM (Corning, Inc.) supplemented with 10% fetal bovine serum (FBS; Biological Industries) and antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin; Gibco; Thermo Fisher Scientific, Inc.). The cells were incubated at 37˚C in a 5% CO_2 incubator and the medium was replaced with fresh medium every 2 days. The experiments were performed in triplicate.

Reverse transcription-quantitative PCR (RT-qPCR). The following groups of ameloblast-like LS8 cells were established: Control; high fluoride, treated with a high fluoride concentration (2.0 mM F^-;Sigma-Aldrich; Merck KGaA); TGF-β1, treated with 10 ng/ml TGF-β1 (Peprotech Inc.); and FT, treated with 2.0 mM F^- + 10 ng/ml TGF-β1. Cells were plated into six-well plates at a density of $1x10^6$ cells/ml and treated with different conditions as aforementioned for 24 h. Total RNA of different groups were extracted using RNAiso (Takara Bio, Inc.) according to the manufacturer's instructions. cDNA was synthesized using the PrimeScript™ RT reagent kit with gDNA Eraser using the temperature protocols of 37˚C for 15 min and 85˚C for 5 sec (Takara, Bio. Inc.). Mouse-specific primers for GAPDH (47), TGF-β1, ClC-5 and ClC-7, were used in conjunction with SYBR Premix Ex Taq™ II (Takara Bio., Inc.) for RNA measurement. All qPCR reactions were conducted under

Table I. Primer sequences for reverse transcription-quantitative PCR.

Gene	Forward sequence (5'-3')	Reverse sequence 5'-3'
TGF-β1	CTTCAATACGTCAGACATTCGGG	GTAACGCCAGGAATTGTTGCTA
ClC-5	GAGGAGCCAATCCCTGGTGTA	TTGGTAATCTCTCGGTGCCTA
ClC-7	CGCCAGTCTCATTCTGCACT	GAGGATCGACTTCCGGGTC
GAPDH	TGACCTCAACTACATGGTCTACA	CTTCCCATTCTCGGCCTTG

TGF-β1, transforming growth factor-β1; ClC-5, voltage-gated chloride channel 5; ClC-7, voltage-gated chloride channel 7.

the following conditions: Hot start for 30 sec at 95˚C, followed by 40 cycles of 5 sec at 95˚C and 30 sec at 60˚C. The primer sequences used are presented in Table I. In the next step, the target mRNA expression levels were determined using the LightCycler 480 Real-Time PCR System (Roche Diagnostics). The relative expression of target genes was calculated using the $2^{-\Delta\Delta Cq}$ method (48). Each group was set with three repeated wells and their mean values were calculated.

Western blot analysis. Total protein was extracted from 60 mm dishes (Corning, Inc.) containing the ameloblast-like LS8 cells with the seeding density of $2x10^6$ cells/ml and treated as described in the previous section. The protein concentration of each sample was determined with a bicinchoninic acid assay (Beijing Solar Bio Science & Technology Co., Ltd.). To separate TGF-β1, ClC-5 and ClC-7, equal amounts of proteins (40 μg) were loaded onto 10% SDS-PAGE gels and then the proteins were transferred onto PVDF membranes. Membranes were blocked in Tris-buffered saline Tween (hereafter called TBST) with 1% Tween and 5% non-fat dry milk for 1 h at room temperature and the membranes were labelled with antibodies against TGF-β1, ClC-5 and ClC-7 at 4˚C overnight. The probed membranes were washed with TBST three times and incubated with appropriate secondary antibodies for 1 h at room temperature. Protein bands were visualised using the enhanced chemiluminescence (ECL, Thermo Fisher Scientific, Inc.) detection system Amersham Imager 600 (GE Healthcare; Cytiva). The primary antibodies used were all rabbit-derived and were anti-GAPDH at a ratio of 1:500 (cat. no. BA2913; Wuhan Boster Biological Technology Co., Ltd.), anti-TGF-β1 at a ratio of 1:1,000 (cat. no. ab92486; Abcam), anti-ClC-5 at a ratio of 1:1,000 (cat. no. ab188503; Abcam), anti-ClC-7 at a ratio of 1:1,000 (cat. no. ab136016; Abcam). Secondary antibodies used were horseradish peroxidase-labelled-goat-derived anti-rabbit at a ratio of 1:5,000 (cat. no. BA1054; Wuhan Boster Biological Technology Co., Ltd.) and horseradish peroxidase-labelled rabbit-derived anti-goat at a ratio of 1:5,000 (cat. no. BA1060; Wuhan Boster Biological Technology Co., Ltd.).

Intracellular fluoride detection through confocal laser scanning microscopy. LS8 cells were seeded into 35 mm glass bottom dishes (cat. no. P35G-1.5-14-C; MatTek Corporation) at a density of $4x10^5$ cells/dish. After adhesion, cells were treated for 24 h, as described for RT-qPCR, before 10 μl the probe 1 was added to each dish and the dishes maintained at room temperature for 30 min (49). The emitted fluorescence intensity was determined using a confocal laser scanning microscope (model no. LSM 780; Zeiss AG) at 543 nm. The mean fluorescence intensities were calculated by ImageJ 1.52v. Probe 1 was provided by Professor Baocun Zhu (Jinan University, Shandong, China).

Statistical analysis. All values are presented as the mean ± the standard deviation (SD). Western blot and RT-qPCR results were analysed by one-way ANOVA. Multiple comparisons between groups were performed using the Tukey's test. $P<0.05$ was considered statistically significant and $P<0.01$ values was considered highly statistically significant.

Results

Fluoride treatment reduces TGF-β1 expression in LS8 cells. Fig. 1A indicates the relative value (compared with the internal reference GAPDH) of TGF-β1 mRNA expression. In the samples from the high fluoride group, endogenous TGF-β1 mRNA expression levels were significantly lower than those of the control group. Fig. 1B indicates the relative value (compared with the internal reference GAPDH) of TGF-β1 protein expression. Consistent with the RT-qPCR results, endogenous TGF-β1 protein expression in the high fluoride group was significantly lower than that of the control group.

Fluoride increases the expression levels of ClC-5 and ClC-7 in LS8 cells. Fig. 2A indicates the relative values (compared with the internal reference GAPDH) of ClC-5 and ClC-7 mRNA expression levels. In the high fluoride group, ClC-5 and ClC-7 mRNA expression levels were significantly higher than those of the control group. Fig. 2B indicates the relative values (compared with the internal reference GAPDH) of ClC-5 and ClC-7 protein expression. ClC-5 protein expression in the high fluoride group was significantly higher than that in the control group. ClC-7 protein expression in the high fluoride group was higher than that in the control group.

Exogenous TGF-β1 reduces the expression of ClC-5 and ClC-7 in LS8 cells. Fig. 3A illustrates the relative values (compared with the internal reference GAPDH) of ClC-5 and ClC-7 mRNA expression levels. In the high fluoride group the ClC-5 and ClC-7 mRNA expression levels were significantly higher than those of the control group. In the exogenous TGF-β1 group, ClC-5 and ClC-7 mRNA expression levels were significantly lower than those of the control group. In the FT group, ClC-5 and ClC-7 mRNA expression levels were significantly lower

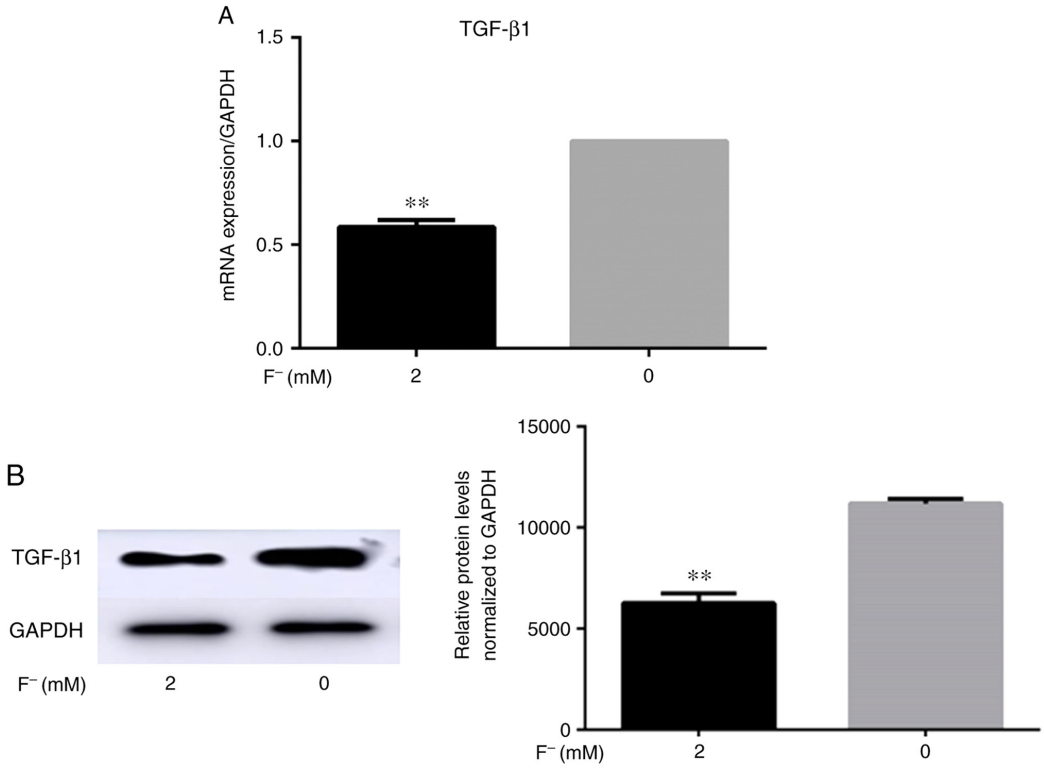

Figure 1. Effect of high fluoride on the TGF-β1 expression in ameloblasts. (A) TGF-mRNA levels and (B) TGF-protein levels were determined in control and high fluoride treated cells. Data are presented as the mean ± the standard deviation of three repeated experiments. **P<0.01 vs. control. TGF-β1, transforming growth factor-β1.

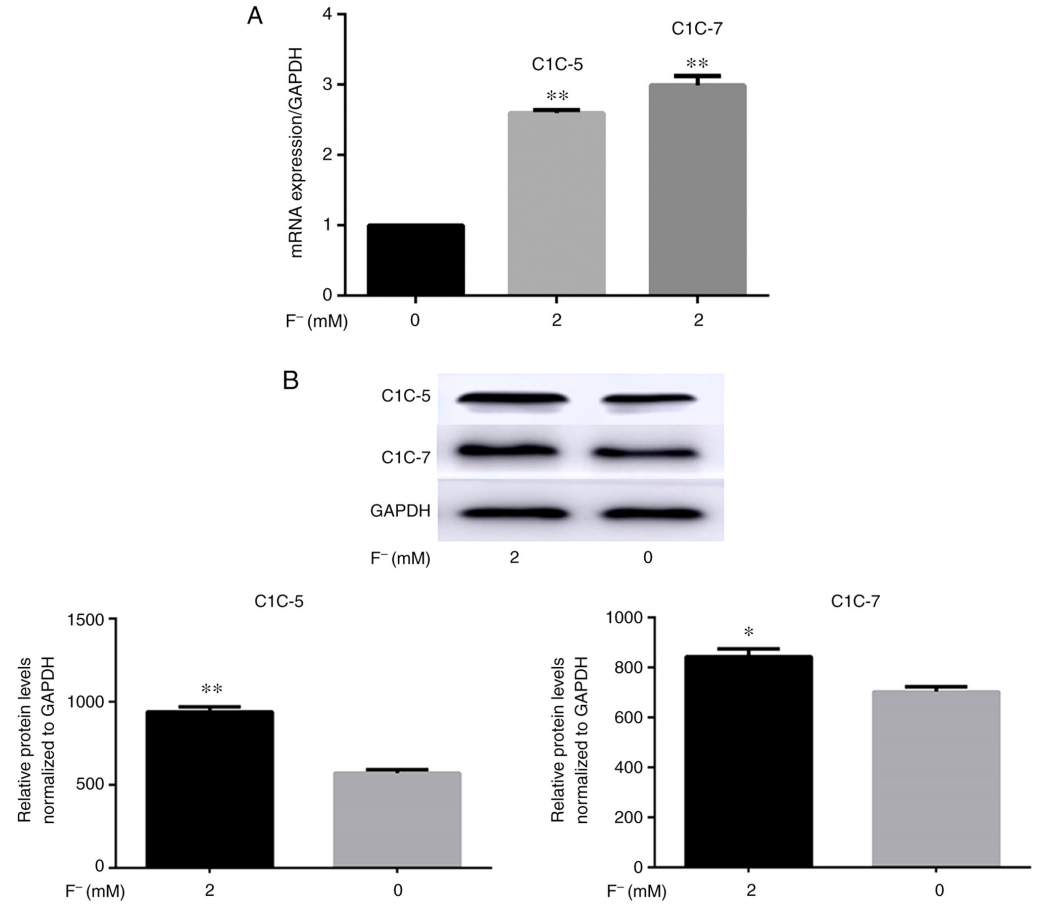

Figure 2. Effects of high fluoride on ClC-5 and ClC-7 expression levels in ameloblasts. Expression levels of ClC-5 and ClC-7 (A) mRNA and (B) protein were determined in control and high fluoride treated cells. Data are presented as the mean ± the standard deviation of three repeated experiments. *P<0.05, **P<0.01 vs. control. TGF-β1, transforming growth factor-β1; ClC-5, voltage-gated chloride channel 5; ClC-7, voltage-gated chloride channel 7.

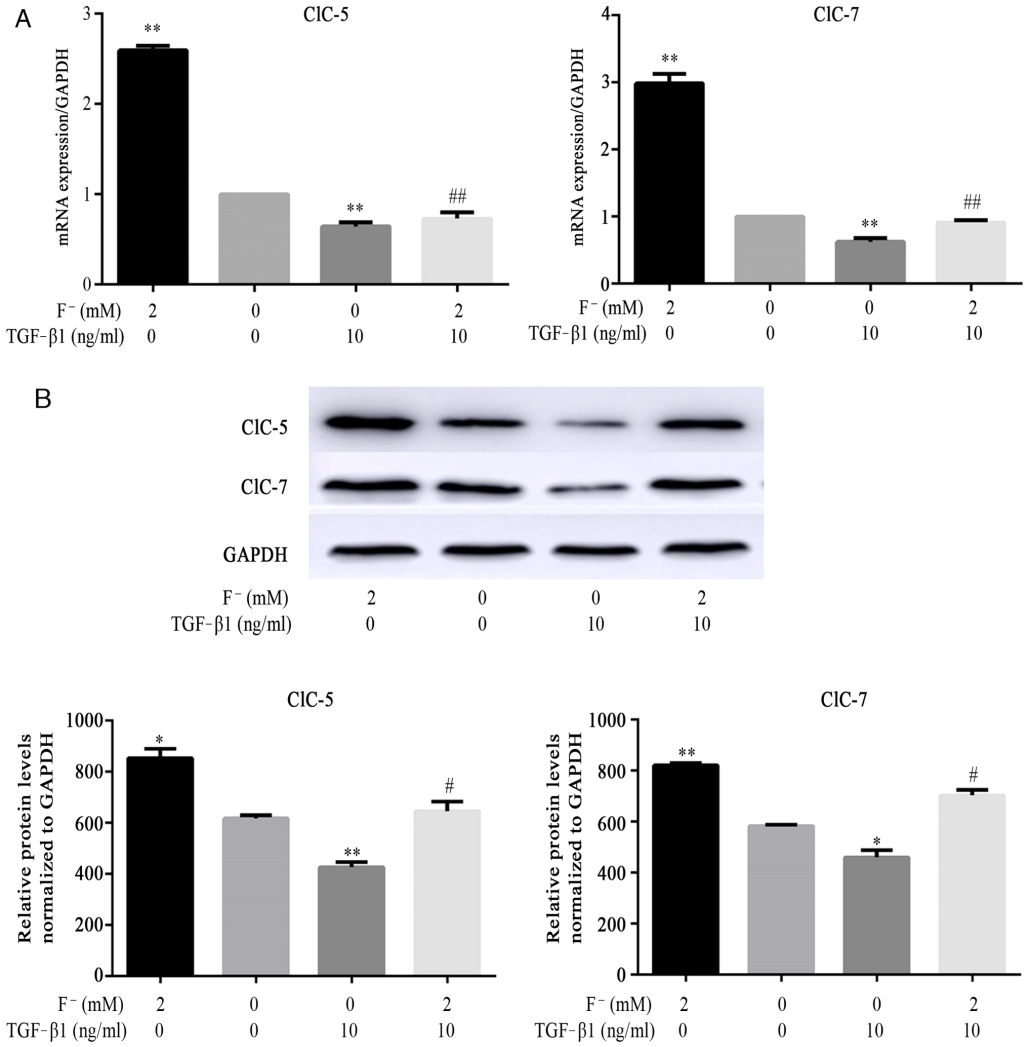

Figure 3. Effects of TGF-β1 on ClC-5 and ClC-7 expression levels in ameloblasts. (A) mRNA and (B) protein levels of ClC-5 and ClC-7 in ameloblasts following treatment with high or low levels of fluoride and exogenous TGF-β1 or a control. *P<0.05, **P<0.01 vs. 0 F + 0 TGF-β1; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. high fluoride. TGF-β1, transforming growth factor-β1; ClC-5, voltage-gated chloride channel 5; ClC-7, voltage-gated chloride channel 7.

than those of the high fluoride group. Fig. 3B illustrates the relative values (compared with the internal reference GAPDH) of ClC-5 and ClC-7 protein expression. In the high fluoride group, ClC-5 protein expression was higher than that of the control group. In the exogenous TGF-β1 group, ClC-5 protein expression was significantly lower than that of the control group. In the FT group, ClC-5 protein expression was lower than that of the high fluoride group. In those in the high fluoride group, ClC-7 protein expression levels were significantly higher than that of the control group. In the exogenous TGF-β1 group, ClC-7 protein expression levels were lower than those of the control group. In the FT group, ClC-7 protein expression levels were lower than those of the high fluoride group.

Exogenous TGF-β1 decreased the fluorescence intensity of LS8 cells. Fig. 4 demonstrates the fluorescence intensity of LS8 cells exposed to different treatments. The fluorescence intensity of the high fluoride group was significantly higher than that of the control group. The fluorescence intensity of the exogenous TGF-β1 group was significantly lower than that of the control group. In the FT group, the fluorescence intensity was significantly lower than that of the high fluoride group.

Discussion

The results of the present study indicated that a high extracellular fluoride concentration had two effects on ameloblasts, an increase in intracellular fluoride concentration and an increase in ClC-5 and ClC-7 expression. Using probe 1, the red fluorescence intensity of intracellular fluoride in the high fluoride group was indicated to be stronger than that in the control group. Fluoride cannot enter ameloblasts directly and must be converted to HF beforehand (8). Protons are released during the process of hydroxyapatite (HA) deposition. Depending on the phosphate precursor, the precipitation of HA releases 8-14 moles of hydrogen ions per mole of HA, which acidifies the enamel matrix (8,13,50). Additionally, a number of *in vitro* and *in vivo* studies have proved that fluoride can accelerate crystal formation and induce hypermineralized lines in secretory enamel (51,52). Crystal growth produces a large number of protons, which can acidify the microenvironment (53). The low pH of the microenvironment promotes the conversion of fluoride to HF. According to the Henderson-Hasselbalch equation, more than 25-fold the amount of HF is produced at pH 6.0, compared with that at pH 7.4 (8,13). Owing to the

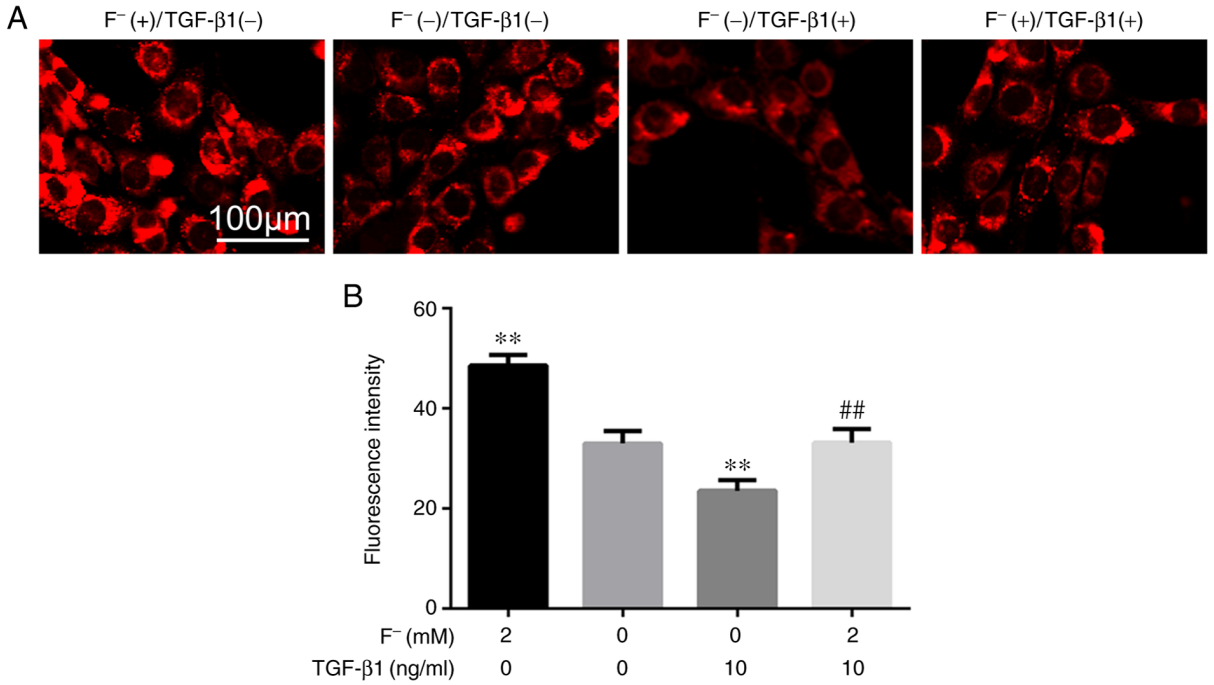

Figure 4. Effects of TGF-β1 on fluorescence intensity in ameloblasts. (A)Fluorescence images and (B) intensities of probe 1 in ameloblasts following treatment with high or low levels of fluoride and exogenous TGF-β1 or a control. . TGF-β1, transforming growth factor-β1; **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. the high fluoride group.

pH gradient, HF may diffuse easily into the cytoplasm from the enamel matrix and revert to fluoride ions in the neutral cytoplasm; consequently, it cannot easily diffuse out of the cell (8,9,13,16). Increased fluoride concentrations in the cytoplasm may induce oxidative stress and endoplasmic reticulum stress, both of which increase the occurrence of dental fluorosis (8,10-12,14,15).

In the present study, probe 1 was employed to determine the intracellular fluoride concentration. Probe 1 is a highly selective ratiometric visual and red-emitting fluorescent dual-channel probe, which can detect fluoride in aqueous solution and living systems (49). In comparison to conventional technology, a ratiometric probe could eliminate most or all ambiguities by self-calibration at two wavelength intensities, which may avoid interference due to instrumental stability, sample environments and probe distribution (17,21,49,54). Additionally, a ratiometric probe may display a large wavelength shift (158 nm) for practical use in ratiometric determination. The 158 nm shift caused by fluoride changes the solution containing probe 1 from yellow to blue (49). Thus probe 1 may serve as a visual probe for fluoride because it can be used to determine fluoride level changes by the naked-eye (49). Additionally, probe 1 could detect fluoride levels on the fluorescence spectrum, due to considerable fluorescence enhancement at 612 nm caused by the addition of fluoride (49). Furthermore, the probe 1 is a rapid analytical method for the detection of fluoride. Previous research suggested that the fluorescence intensity at 612 nm increased with reaction time and then leveled off at a reaction time >~25 min (49), which made probe 1 use easier. On the basis of this finding 30 min was chosen as the time point to assess the fluorescence intensity of fluoride using probe 1 in the present study.

The second effect of fluoride on ameloblasts an increase in ClC-5 and ClC-7 expression. As voltage-gated chloride channels, ClC-5 and ClC-7 are responsible for exchanging Cl^- and H^+ to maintain cell acidification. High expression levels of ClC-5 promote the influx of fluoride into the cytoplasm. ClC-7 localizes in vesicles of the endocytotic-lysosomal pathway in different cell types (30). It has been indicated that during the maturation stage of ameloblasts, the highest levels of ClC-7 are localized in ameloblast vesicles (33,55). Thus, the high expression of ClC-7 promotes the influx of fluoride into the endosome/lysosome. This may induce toxicity in ameloblasts, thereby leading to dental fluorosis (8,13). CFTR stimulates the transport activity of Slc26a members, which leads to bicarbonate efflux (37,56,57). Additionally, CFTR is permeable to bicarbonate (37,58,59). Although the results of previous studies have indicated that CFTR is more permeable to Cl^- than to bicarbonate, studies have revealed that CFTR may be responsible for more than 50% of the total bicarbonate efflux in pancreatic duct cells (37,53,58). The low pH caused by HA deposition may lead to an increase in expression of the electrolyte transporter responsible for the efflux of bicarbonate (60,61). The released bicarbonate may neutralize protons to produce a microenvironment that favors crystal nucleation. However, in the present study, no significant changes in CFTR were identified. This may be due to the use of different cell types or low expression of CFTR in LS8 cells. Further research is needed to better understand the mechanism underlying CFTR expression. Duan *et al* (38) found that excess fluoride inhibited endocytic activity of ameloblasts through the CFTR chloride channel or other chloride channels. CFTR siRNA and CFTR-specific channel inhibitor were employed to disturb the function of CFTR, which could examine the effects on the transport activity in presence of fluoride (38). Thus, future work will assess the effects of ClCs inhibitors

or silencing with siRNAs on the expression and transport activity of ameloblasts.

TGF-β1 is distributed in most cells and is activated through the release of latency-associated protein 1 and latent TGF-β-binding protein 1 (62-64). Active TGF-β1 may activate and affect organic homeostasis by binding to the transforming growth factor-β receptor 1 (TGF-βR1) through the TGF-β1-Smad signal pathway. Previous studies have shown that fluoride may repress the expression of TGF-β1 (13,41,65,66) and the present study confirmed this result. In the present study, fluoride significantly reduced the expression levels of TGF-β1 in ameloblasts at the gene and protein levels. TGF-β1 may also impair the expression and function of chloride ion channels in other epithelial cells, including alveolar, bronchial, vas deferens and nasal polyp epithelial cells (43-46). To investigate the relationship between TGF-β1 and chloride ion channels, exogenous TGF-β1 was added to the culture medium. The present results suggested a largely negative correlation between them. Exogenous TGF-β1 significantly reduced the expression levels of ClC-5 and ClC-7 in comparison with a control. When TGF-β1 and high fluoride levels were added to the medium at the same time, the increase in the expression levels of ClC-5 and ClC-7 caused by fluoride was markedly reduced compared with high fluoride treatment alone. These results suggested that the addition of exogenous TGF-β1 may compensate for the changes in ClC-5 and ClC-7 caused by the decrease of endogenous TGF-β1 induced by fluoride. With the inhibition of ClC-5 and ClC-7 expression, the red fluorescence intensity of intracellular fluoride was weaker compared with that of the control group.

Both the decreased pH and the highly expressed ClC-5 and ClC-7 levels caused by the high fluoride concentration accelerated the accumulation of fluoride in ameloblasts. If the buffering capacity of ameloblasts is overwhelmed by an excessive amount of fluoride, hypomineralization occurs and may cause dental fluorosis. The present study may provide a new perspective for the prevention of dental fluorosis through TGF-β-mediated reduction of fluoride accumulation. The present study assessed the effect of exogenous TGF-β1 on preventing the inflow of fluoride to ameloblasts through regulation of ClC-5 and ClC-7 for 24 h. A longer time course will be utilized in future studies. Additionally, further studies are required to investigate the precise mechanism by which the ions are transported. Studies will also be conducted to confirm this hypothesis *in vivo*.

In conclusion, with the use of probe 1, it was demonstrated that exogenous TGF-β1 may prevent the accumulation of intracellular fluoride through the regulation of ClC-5 and ClC-7 in ameloblasts under high extracellular fluoride concentrations.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DZ and XD contributed to the conception and design of the present study. MJ, XH and JS conducted all the experiments. MJ and XD drafted the manuscript and revised it critically for important intellectual content. MJ, XH and JS interpreted and analyzed the data. MJ and XH were responsible for assessing the authenticity of all the raw data. All authors read and approve the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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