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Fluoride Affects Calcium Homeostasis by Regulating Parathyroid Hormone, PTH-Related Peptide, and Calcium-Sensing Receptor Expression

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Abstract Parathyroid hormone (PTH), PTH-related peptide (PTHrP), and calcium-sensing receptor (CaSR) play important roles in maintaining calcium homeostasis. Here, we study the effect of fluoride on expression of PTH, PTHrP, and CaSR both in vitro and in vivo. MC3T3-E1 cells and Sprague-Dawley rats were treated with different concentrations of fluoride. Then, the free calcium ion concentration in cell culture supernatant and serum were measured by biochemical analyzer. The expression of PTH, PTHrP, and CaSR was analyzed by qRT-PCR and Western blot. We found that the low dose of fluoride increased ionized calcium (i[Ca^{2+}]) and the high dose of fluoride decreased i[Ca^{2+}] in cell culture supernatant. The low dose of fluoride inhibited the PTH and PTHrP expression in MC3T3-E1 cells. The high dose of fluoride improved the PTHrP expression in MC3T3-E1 cells. Interestingly, we found that NaF decreased serum i[Ca^{2+}] in rats. Fluoride increased CaSR expression at both messenger RNA (mRNA) and protein levels in MC3T3-E1 cells and rats. The expression of PTHrP protein was inhibited by fluoride in rats fed regular diet and was increased by fluoride in rats fed low-calcium diet. Fluoride also increased the expression of PTH, NF-kappaB ligand (RANKL), and osteoprotegerin (OPG) in rats. The ratio of RANKL/OPG in rats fed low-calcium food in presence or absence of fluoride was significantly increased. These results indicated that fluoride might be able to affect calcium homeostasis by regulating PTH, PTHrP, and CaSR.

Keywords Fluoride · Calcium homeostasis · Parathyroid hormone · PTH-related peptide · Calcium-sensing receptor

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Introduction

Fluoride is a trace element that plays an important role in both bone formation and homeostasis of bone mineral metabolism [1]. Low level of fluoride intake can prevent dental caries. However, prolonged and excessive ingestion of fluoride can cause fluorosis, which affects both teeth and bones. The dental fluorosis is characterized by tooth discoloration and enamel damage. The skeletal fluorosis can cause the most serious consequence, such as crippling, osteoporosis, and osteosclerosis. The previous studies have suggested that fluoride can cause cytotoxicity in a concentration-dependent manner [2]. Meanwhile, the long-term exposure to this element affects the amount and catalytic properties of many enzymes. Low concentration of fluoride stimulates the osteoblast proliferation [3, 4]. At high dose, the toxic effect of fluoride includes inflammatory reactions [5], oxidative stress [6, 7], and DNA damage [8]. Fluoride induces apoptosis by alteration in expression of both Bax and Bcl-2 in osteoblast [3, 9].

The interaction between fluoride and calcium has been reported for years [10]. The calcium ion is an essential intracellular messenger and modulates a diverse range of cellular physiological functions, including muscle contraction[11], gene transcription [12], and cell proliferation[13]. Recent studies indicated that fluoride induces the intracellular calcium ion release and results in the elevation of reactive oxygen

reactions that relates to the cytotoxicity [14]. The increased intracellular calcium ion is associated with the fluoride-induced apoptosis [15]. Chronic fluoride ingestion affects calcium ion homeostasis by reduction of the amount of calcium pump proteins [16]. In contrast, fluoride leads to increased cytosolic calcium concentrations in proximal tubules [17], fibroblasts [18], and osteoblasts [19]. However, the mechanism of effect of fluoride on calcium homeostasis has not been sufficiently established.

The extracellular fluid calcium concentration is tightly regulated by parathyroid hormone (PTH), a hormone that can stimulate bone resorption and release calcium from bone, stimulate calcium reabsorption from the renal tubules, and increase production of $1,25(OH)_2D_3$ in kidney to enhance intestinal absorption of calcium [20]. PTH-related peptide (PTHrP), a peptide produced by tumors with close homology in the N-terminal sequence to PTH itself [21], was originally isolated as a causal factor for hypercalcemia of malignancy (HM). PTHrP has been shown to play a critical role in bone formation by promoting survival and differentiation of osteoblasts [22, 23]. The interaction between fluoride and PTH and/or PTHrP has not been sufficiently investigated.

We previous demonstrated that L-type calcium channels are important in fluoride-related calcium ion homeostasis in osteoblasts [24]. In the present study, we investigate the role of fluoride in the expression of PTH, PTHrP, and calcium-sensing receptor (CaSR).

Materials and Methods

Measurement of Free Calcium Ion Concentration

The osteoblastic cell line MC3T3-E1 was obtained from Cell Bank (Shanghai, China). MC3T3-E1 cells were cultured in alpha minimum essential medium (Gibco Laboratories, Grand Island, NY) supplemented with 10 % heat-inactivated fetal bovine serum and 100 U/mL of penicillin and 100 μg/mL of streptomycin (Sigma Chemicals, St. Louis, MO). The cells were maintained at 37 °C in a humidified incubator in an atmosphere of 5 % CO_2-95 % air and passaged at a 1:5 ratio with trypsin every 3–5 days. Cells (1×10^4) were plated into 96-well plates and treated with different doses of NaF for 48 h. Then, the supernatant was collected and the concentration of free calcium ion was measured with biochemical analyzer (CS-400B, Dirui, China).

Animals and Treatment

Forty Sprague-Dawley male rats were purchased from Beijing Laboratory Animal Center in China. All procedures involving animals were carried out in strict accordance with the international standards of animal care guidelines and were approved by the local Care of Experimental Animals Committee. Rats were randomly divided into four groups. Each experimental group contained ten rats. Control group was feed regular food and water, and others were raised by either regular food and 100 mg/L NaF water, or low-calcium food and regular water, or low-calcium food and 100 mg/L NaF water for 2 months, respectively. The food and water were renewed every day during the experiment. At the end of the experiment, long bones (femoral and tibiofibula) were collected and the concentration of free calcium ion in serum was measured with biochemical analyzer as above (CS-400B, Dirui, China).

RNA Isolation and qRT-PCR

MC3T3-E1 cells were cultured in 5 % FCS for 24 h and then treated with α-MEM (5 % calf serum) containing fluoride at a dose of 0, 2, 5, and 10 mg/L for 48 h. The bone samples from animal experiments were immersed in liquid nitrogen and pulverized. Total RNA of both cells and bones was extracted using TRIzol reagent (Invitrogen Inc., USA) according to the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized from l μg of total RNA in the present of oligo $(dT)_{18}$ primer and reverse transcriptase (Takara, Japan). Quantitative PCR was performed on ABI 7500 real-time PCR system (Applied Biosystems, CA). cDNA template, primers, and SYBR Premix Ex Tag (Takara, Japan) in a total of 20 μL were applied to the following PCR programs: 95 °C preheating for 1 min, followed by 40 cycles of 95 °C for 15 s (denaturation), 60 °C for 20 s (annealing), and 72 °C for 32 s (elongation). The real-time PCR primer sequences are listed in Table 1. Gene expression was analyzed using the comparative threshold cycle (ΔΔCt) method [25], and relative expression levels were quantified by normalizing to β-actin expression.

Protein Isolation and Western Blot

MC3T3-E1 cells and pulverized bones were lysed with RIPA buffer (Beyotime, China). The protein concentration of the samples was measured with a bicinchoninic acid protein assay kit (BCA, Beyotime, China). For Western blot, an equivalent amount of protein from cells or bones was mixed with 5× loading buffer (4:1) and boiled at 100 °C for 10 min. Then, the samples were separated by SDS-PAGE and transferred to PVDF membranes (PALL, USA). Membranes were incubated overnight at 4 °C with primary antibodies (β-actin, 1:5000, Proteintech; PTH, 1:200, Santa Cruz; PTHrP, 1:200, Santa Cruz; CaSR, 1:100, Santa Cruz; osteoprotegerin (OPG), 1:100, Santa Cruz; RANKL, 1:600, Epitomics). After three washes with TBS/0.1 % Tween 20 for 5 min, the membranes were incubated with secondary antibodies for 1 h at room temperature [26]. The immune blot signals were visualized using the EasySee Western Blot Kit (TransGen, China). The protein expression levels were determined by densitometric scanning (Tanon-1600 gel image system, Shanghai, China).

Table 1 The real-time PCR primer sequences

Gene name	Accession number	Sequences
CaSR	NM_013803.2	Forward: 5′-GAAGCCAAGATACCCACC AG-3′
		Reverse: 5′-CGTGTAGAGCCAGATGAT GC-3′
PTH	BC099456.1	Forward: 5′-GAAACCCGTGAGGAAGA-3′
		Reverse: 5′-TTGCCATCAACAAGGAC-3′
PTHrP	NM_012636.1	Forward: 5′-CAGACGACGAGGGCAGAT-3′
		Reverse: 5′-GACCGAGTCCTTCGCTTT-3′
β-Actin	X03672	Forward: 5′-GTCAGGTCATCACTATCG GCAAT-3′
		Reverse: 5′-AGAGGTCTTTACGGATGT CAACGT-3′

Statistical Analysis

Statistical analyses of the above results were performed by a one-way analysis of variance (ANOVA) by using the SPSS program (version 13.0) for windows (SPSS, Chicago, IL, USA). When ANOVA indicated a significant difference, the further analysis was performed by using the Fisher protected least significant difference (PLSD). Differences are considered statistically significant if $p<0.05$.

Results

Fluoride Affected the Concentration of Free Calcium Ion in MC3T3-E1 Cell Culture Supernatant and Serum in Rats

To determine the function of fluoride on free calcium ion, we either treated the MC3T3-E1 cells with different concentration of fluoride or fed the rats with or without fluoride. The concentration of ionized calcium (i[Ca^{2+}]) in cell culture supernatant and serum was measured with biochemical analyzer. As shown in Fig. 1a, the concentration of i[Ca^{2+}] in cell culture supernatant was increased at 2 mg/L dose of fluoride and was decreased at high dose of fluoride (5 and 10 mg/L), as compared with control group. Interestingly, we found that the concentration of i[Ca^{2+}] in serum was decreased in rats fed 100 mg/L NaF in presence or absence of calcium supplement in food (Fig. 1b).

Quantitation of CaSR, PTH, and PTHrP mRNA and Protein Expression

To examine the effect of fluoride on calcium receptors, we studied the expression of CaSR by both Western blot and qRT-PCR. As demonstrated in Table 2 and Fig. 2, fluoride increased CaSR expression at both messenger RNA (mRNA) and protein levels in MC3T3-E1 cells and rats. We next studied the expression of PTH in MC3T3-E1 cells and long bones. As shown in Table 3 and Fig. 3, the PTH expression was decreased at both mRNA and protein levels in MC3T3-E1 cells after fluoride treatment at 2 and 5 mg/L. High dose of fluoride decreased the expression of PTH both at mRNA and protein level in MC3T3-E1 cells. The Western blot in bone indicated that PTH was increased in rats fed fluoride (Fig. 3d). Table 4 and Fig. 4 show the function of fluoride on PTHrP expression. When compared with control group, the low dose of fluoride (2 mg/L) decreased the expression of PTHrP protein and the high dose of fluoride (10 mg/L) increased the expression of PTHrP protein in MC3T3-E1 cells. The fluoride with regular food inhibited the expression of PTHrP protein, while fluoride with low-calcium food increased the expression of PTHrP protein.

The Effect of Fluoride on RANKL and OPG

To determine the effect of fluoride on osteoclastogenesis, we examined the expression of OPG and RANKL in rats after

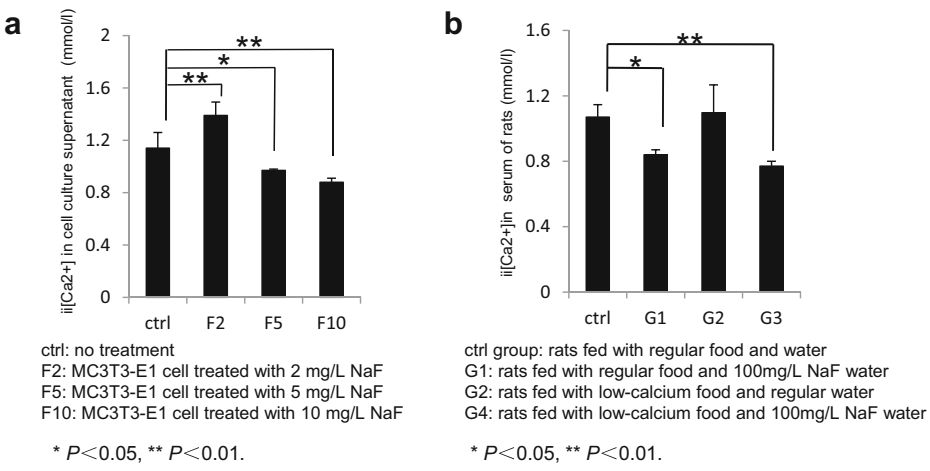

Fig. 1 The concentration of free calcium in cell culture supernatant and serum. **a** The MC3T3-E1 cells were treated with different doses of NaF for 48 h. Then, the supernatant was collected and biochemical analyzer was used to measure the concentration of free calcium ion. **b** The concentration of free calcium ion in serum from rats fed fluoride

ctrl: no treatment
F2: MC3T3-E1 cell treated with 2 mg/L NaF
F5: MC3T3-E1 cell treated with 5 mg/L NaF
F10: MC3T3-E1 cell treated with 10 mg/L NaF

* $P<0.05$, ** $P<0.01$.

ctrl group: rats fed with regular food and water
G1: rats fed with regular food and 100mg/L NaF water
G2: rats fed with low-calcium food and regular water
G4: rats fed with low-calcium food and 100mg/L NaF water

* $P<0.05$, ** $P<0.01$.

Table 2 The expression level of CaSR mRNA in MC3T3-E1 cells by RT-PCR

ctrl	1.26±0.81
F2	14.15±3.33**
F5	9.97±1.68**
F10	16.7±2.26**

*$P<0.05$; **$P<0.01$

Table 3 The expression level of PTH mRNA in MC3T3-E1 cells by RT-PCR

ctrl	16.4±2.83
F2	1.67±0.48**
F5	0.56±0.25**
F10	27.27±4.72*

*$P<0.05$; **$P<0.01$

treated with or without fluoride. The expression levels of OPG and RANKL were increased in rats with regular food and 100 mg/L NaF water, low-calcium food and regular water, and low-calcium food and 100 mg/L NaF water (Fig. 5). The ratio of RANKL/OPG in rats fed low-calcium food in presence or absence of fluoride was significantly increased, as compared with control group (Fig. 5).

Discussion

Previous studies have demonstrated that fluoride increases metabolic turnover of bones and affects the homeostasis of bone mineral metabolism by stimulating osteoblast proliferation, although the mechanism is not yet understood [1, 3, 27–29]. High fluoride ingestion can cause hypocalcemia in fluorotic patients by increased calcitonin, and then, PTH levels were raised in serum to maintain calcium levels.

In the present study, we investigated the role of fluoride in calcium homeostasis and osteogenic transcription factor expressions both in vitro and in vivo. It has been demonstrated that intracellular calcium concentration is 10,000 to 100,000 times less than extracellular calcium concentration [30]. In plasma, calcium exists in three different forms: ionized or the biologically active form, protein-bound calcium, and complexed to phosphate and citrate [31]. Our results showed that the concentration of free calcium ion was slightly increased at low dose of fluoride and decreased at high dose of fluoride in cell culture supernatant. Previous studies showed that low dose of fluoride does not alter viability in MC3T3-E1 cells [32, 33] and high dose of fluoride increases PTH secretion [39]. Our previous study indicated that PTH and PTHrP play a critical role in anabolic effect of fluoride on bone turnover in osteoblasts [40]. Fluoride affects PTH and PTHrP expression in our study. These results indicate that fluoride might affect calcium ion concentration by regulating the secretion of PTH

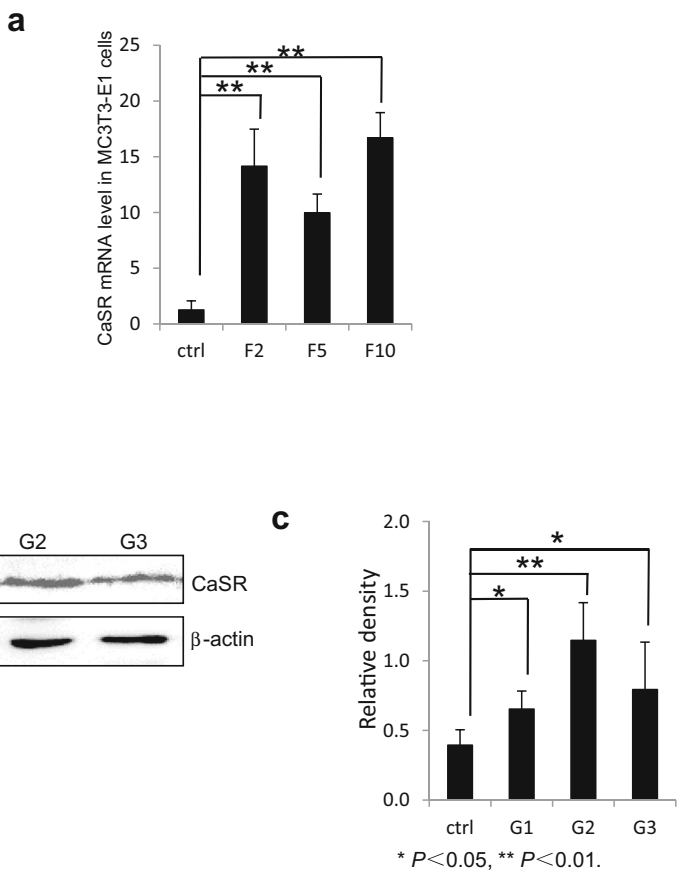

Fig. 2 Quantitation of CaSR mRNA and protein expression. **a** The expression level of CaSR mRNA in MC3T3-E1 cells by RT-PCR. **b** The expression level of CaSR protein in rats by Western blot analysis with β-actin as loading control. **c** The graph indicates the relative density of CaSR protein in rats

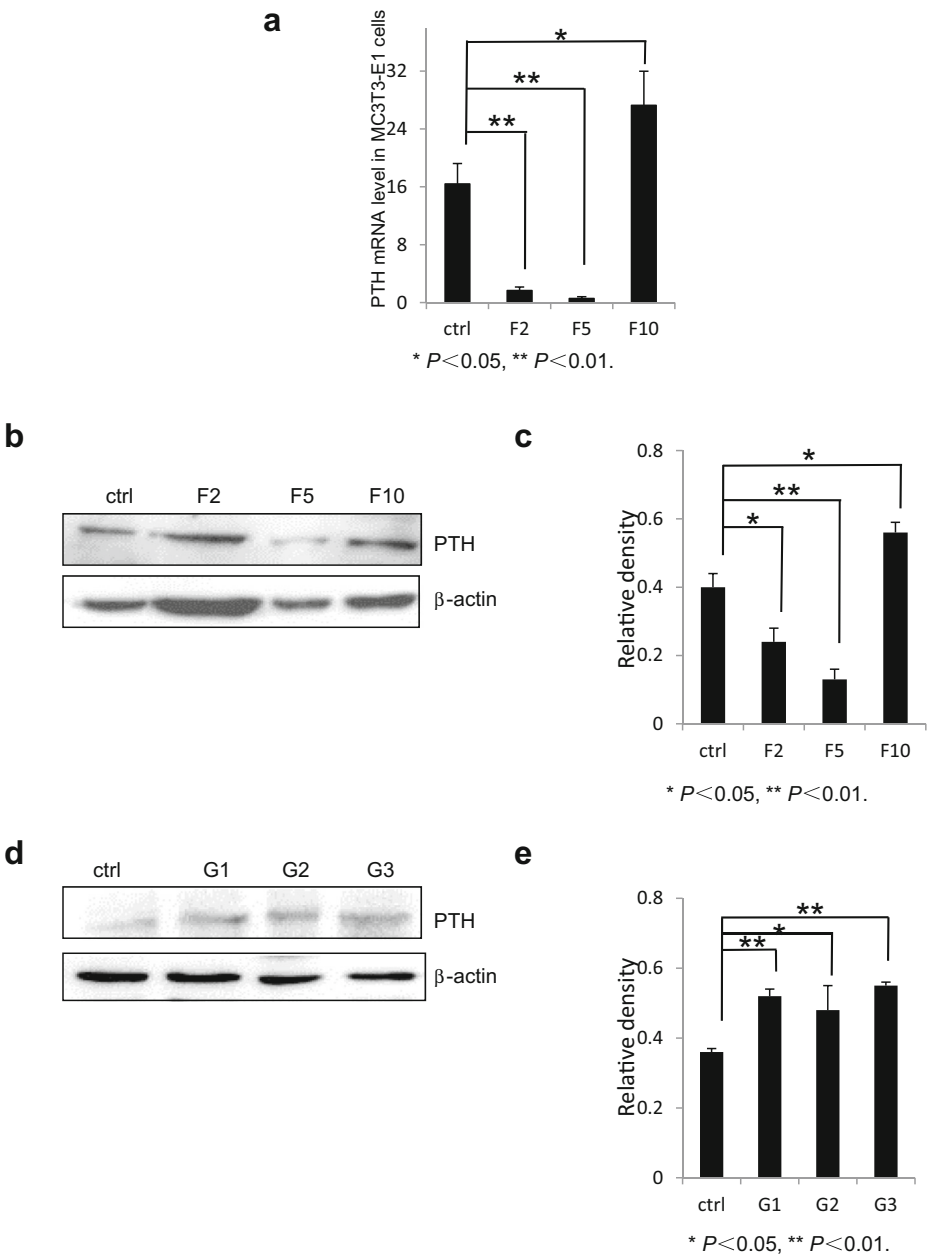

Fig. 3 Quantitation of PTH mRNA and protein expression. **a** The expression level of PTH mRNA in MC3T3-E1 cells by RT-PCR. **b** The expression level of PTH protein in MC3T3-E1 cells by Western blot analysis with β-actin as loading control. **c** The graph indicates the relative density of PTH protein in MC3T3-E1 cells. **d** The expression level of PTH protein in rats by Western blot analysis with β-actin as loading control. **e** The graph indicates the relative density of PTH protein in rats

and PTHrP. The extracellular CaSR is a G protein-coupled receptor that is activated by extracellular calcium ion and plays an important role in regulating calcium homeostasis [34, 35]. The CaSR is found in multiple tissues including the parathyroid gland, thyroid, kidney, and bone [35]. In the parathyroid cell, CaSR is coupled to a G protein belonging to the Gq/G11 subfamily [36]. The CaSR maintains a stable ionized calcium concentration in the blood by modulating PTH secretion. Additionally, the signaling through the CaSR suppresses parathyroid gene transcription and cell proliferation through posttranslational mechanism [37, 38]. Our study showed that fluoride led to increase CaSR expression at dose-dependent manner at both the mRNA and protein levels in MC3T3-E1 cells and rats. Therefore, fluoride plays critical roles in calcium homeostasis maintenance. Fluoride upregulates the CaSR expression by modulating the extracellular calcium, then leads to downregulate the PTH and PTHrP expression. But high expression level of PTH and PTHrP were showed in our results.. Further studies should be done to investigate if there are fluoride receptors in osteoblast and activate downstream transcription factors and stimulate the PTH and PTHrp expression.

Table 4 The expression level of PTHrP mRNA in rats by RT-PCR

ctrl	10.78±1.87
G1	3.02±0.49*
G2	47.95±8.31**
G3	114.99±19.92**

*$P<0.05$; **$P<0.01$

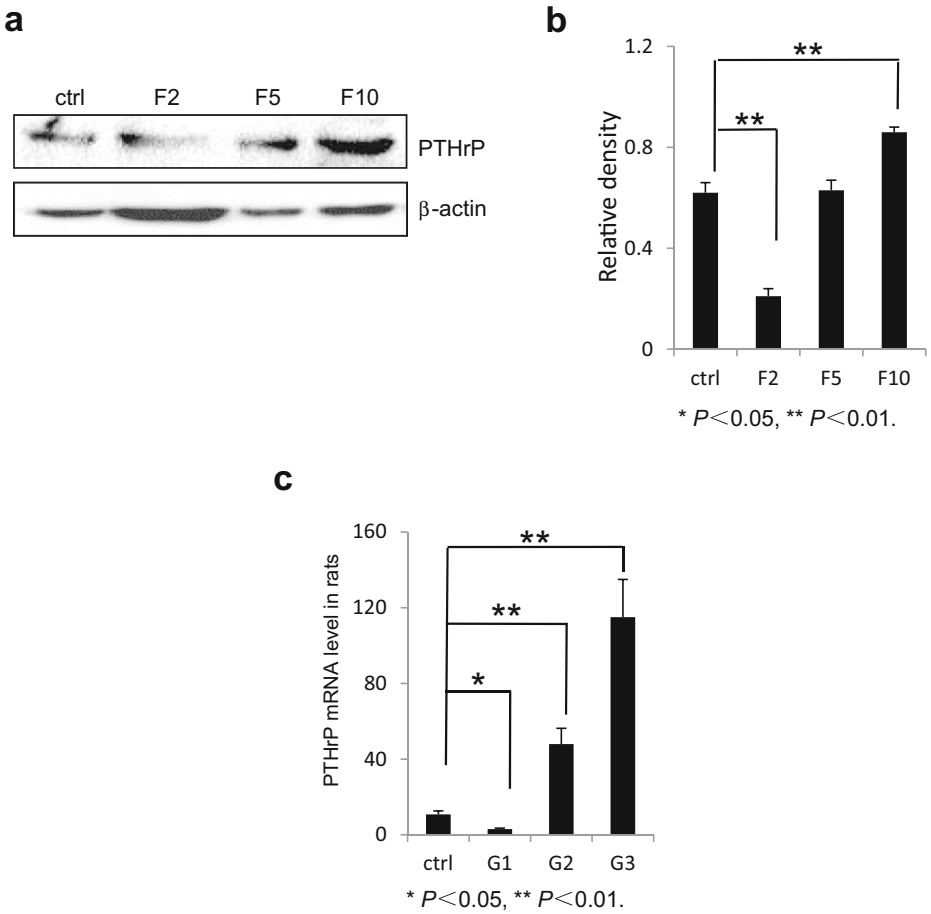

Fig. 4 Quantitation of PTHrP mRNA and protein expression. **a** The expression level of PTHrP protein in MC3T3-E1 cells by Western blot analysis with β-actin as loading control. **b** The graph indicates the relative density of PTHrP protein in MC3T3-E1 cells. **c** The expression level of PTHrP mRNA in rats by RT-PCR

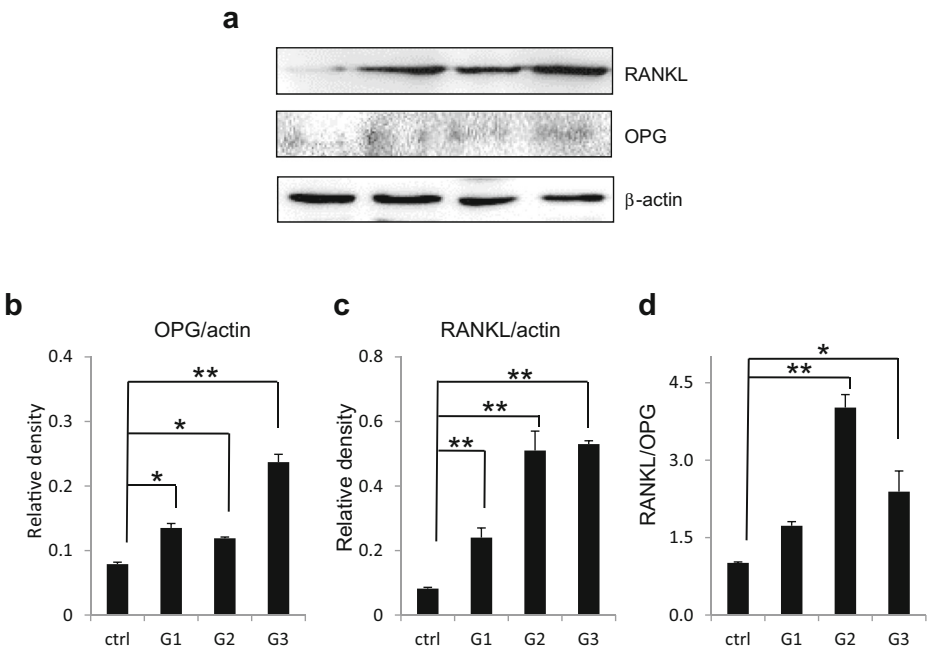

Fig. 5 Expression of RANKL and OPG in rats in presence of fluoride. **a** The expression level of RANKL and OPG protein by Western blot analysis with β-actin as loading control. **b**, **c** The graphs indicate the relative density of OPG and RANKL protein, respectively. **d** The ratio of RANKL and OPG

The receptor activators of RANKL and OPG are key molecules in bone remodeling by regulating differentiation and activation of osteoclasts. Osteoblasts express RANKL which initiates osteoclastogenesis by activating a variety of downstream signaling pathways such as mitogen-activated protein kinases, NF-κB, AP-1, c-fos, and NFATc1 [41]. The ratio of OPGL/OPG determines the delicate balance between bone resorption and synthesis. The previous study indicated that the RANKL/OPG system seems to be affected directly or indirectly by fluoride [42]. In our study, fluoride increased RANKL and OPG expression. These results indicated that the calcium and high fluoride enhanced the activity of osteoclasts and bone remodeling.

In summary, we have presented data that indicate that fluoride has an effect on the regulation of CaSR expression by modulating the extracellular calcium and then regulates the secretion of PTH and PTHrP. CaSR is important in modulating the calcium ion concentration in the present of fluoride. But, the mechanism is not clear and needs further studies.

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Conflict of Interest All authors declare that there are no conflicts of interest.

References

1. Mousny M, Banse X, Wise L, Everett ET, Hancock R et al (2006) The genetic influence on bone susceptibility to fluoride. Bone 39: 1283–1289
2. Nguyen Ngoc TD, Son YO, Lim SS, Shi X, Kim JG et al (2012) Sodium fluoride induces apoptosis in mouse embryonic stem cells through ROS-dependent and caspase- and JNK-mediated pathways. Toxicol Appl Pharmacol 259:329–337
3. Qu WJ, Zhong DB, Wu PF, Wang JF, Han B (2008) Sodium fluoride modulates caprine osteoblast proliferation and differentiation. J Bone Miner Metab 26:328–334
4. Sherwood IA (2010) Fluorosis varied treatment options. J Conserv Dent 13:47–53
5. Salgado-Bustamante M, Ortiz-Perez MD, Calderon-Aranda E, Estrada-Capetillo L, Nino-Moreno P et al (2010) Pattern of expression of apoptosis and inflammatory genes in humans exposed to arsenic and/or fluoride. Sci Total Environ 408:760–767
6. Flora SJ, Mittal M, Mishra D (2009) Co-exposure to arsenic and fluoride on oxidative stress, glutathione linked enzymes, biogenic amines and DNA damage in mouse brain. J Neurol Sci 285:198–205
7. Basha PM, Madhusudhan N (2010) Pre and post natal exposure of fluoride induced oxidative macromolecular alterations in developing central nervous system of rat and amelioration by antioxidants. Neurochem Res 35:1017–1028
8. Zhang M, Wang A, Xia T, He P (2008) Effects of fluoride on DNA damage, S-phase cell-cycle arrest and the expression of NF-kappaB in primary cultured rat hippocampal neurons. Toxicol Lett 179:1–5
9. Yang S, Wang Z, Farquharson C, Alkasir R, Zahra M et al (2011) Sodium fluoride induces apoptosis and alters bcl-2 family protein expression in MC3T3-E1 osteoblastic cells. Biochem Biophys Res Commun 410:910–915
10. Peters JH, Greenman L, Danowski TS (1948) Beneficial effects of calcium chloride in fluoride poisoning. Fed Proc 7:92
11. Berchtold MW, Brinkmeier H, Muntener M (2000) Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 80:1215–1265
12. Johnson CM, Hill CS, Chawla S, Treisman R, Bading H (1997) Calcium controls gene expression via three distinct pathways that can function independently of the Ras/mitogen-activated protein kinases (ERKs) signaling cascade. J Neurosci 17:6189–6202
13. Apati A, Janossy J, Brozik A, Bauer PI, Magocsi M (2003) Calcium induces cell survival and proliferation through the activation of the MAPK pathway in a human hormone-dependent leukemia cell line, TF-1. J Biol Chem 278:9235–9243
14. Xu Z, Xu B, Xia T, He W, Gao P et al (2013) Relationship between intracellular Ca(2)(+) and ROS during fluoride-induced injury in SH-SY5Y cells. Environ Toxicol 28:307–312
15. Kubota K, Lee DH, Tsuchiya M, Young CS, Everett ET et al (2005) Fluoride induces endoplasmic reticulum stress in ameloblasts responsible for dental enamel formation. J Biol Chem 280:23194–23202
16. Borke JL, Whitford GM (1999) Chronic fluoride ingestion decreases 45Ca uptake by rat kidney membranes. J Nutr 129:1209–1213
17. Dominguez JH, Garcia JG, Rothrock JK, English D, Mann C (1991) Fluoride mobilizes intracellular calcium and promotes Ca2+ influx in rat proximal tubules. Am J Physiol 261:F318–327
18. Kawase T, Ishikawa I, Suzuki A (1988) The calcium-mobilizing action of low concentrations of sodium fluoride in single fibroblasts. Life Sci 42:1253–1257
19. Zerwekh JE, Morris AC, Padalino PK, Gottschalk F, Pak CY (1990) Fluoride rapidly and transiently raises intracellular calcium in human osteoblasts. J Bone Miner Res 5(Suppl 1):S131–136
20. Juppner H, Abou-Samra AB, Freeman M, Kong XF, Schipani E et al (1991) A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science 254:1024–1026
21. Mundy GR, Edwards JR (2008) PTH-related peptide (PTHrP) in hypercalcemia. J Am Soc Nephrol 19:672–675
22. Martin TJ (2005) Osteoblast-derived PTHrP is a physiological regulator of bone formation. J Clin Invest 115:2322–2324
23. Miao D, He B, Jiang Y, Kobayashi T, Soroceanu MA et al (2005) Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1-34. J Clin Invest 115:2402–2411
24. Duan XQ, Zhao ZT, Zhang XY, Wang Y, Wang H et al (2014) Fluoride affects calcium homeostasis and osteogenic transcription factor expressions through l-type calcium channels in osteoblast cell line. Biol Trace Elem Res 162:219–226
25. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408
26. Hu Q, Fu H, Ren T, Wang S, Zhou W et al (2008) Maternal low-level lead exposure reduces the expression of PSA-NCAM and the activity of sialyltransferase in the hippocampi of neonatal rat pups. Neurotoxicology 29:675–681
27. Yildiz M, Oral B (2006) The effect of pregnancy and lactation on bone mineral density in fluoride-exposed rats. Toxicol Ind Health 22: 217–222
28. Everett ET (2011) Fluoride's effects on the formation of teeth and bones, and the influence of genetics. J Dent Res 90:552–560
29. Isa ZM, Schneider GB, Zaharias R, Seabold D, Stanford CM (2006) Effects of fluoride-modified titanium surfaces on osteoblast proliferation and gene expression. Int J Oral Maxillofac Implants 21:203–211
30. Drummond IA, Lee AS, Resendez E Jr, Steinhardt RA (1987) Depletion of intracellular calcium stores by calcium ionophore

A23187 induces the genes for glucose-regulated proteins in hamster fibroblasts. J Biol Chem 262:12801–12805
31. Jorde R, Sundsfjord J, Fitzgerald P, Bonaa KH (1999) Serum calcium and cardiovascular risk factors and diseases: the Tromso study. Hypertension 34:484–490
32. Slompo C, Buzalaf CP, Damante CA, Martins GM, Hannas AR et al (2012) Fluoride modulates preosteoblasts viability and matrix metalloproteinases-2 and -9 activities. Braz Dent J 23:629–634
33. Wei M, Duan D, Liu Y, Wang Z, Li Z (2014) Autophagy may protect MC3T3-E1 cells from fluoride-induced apoptosis. Mol Med Rep 9: 2309–2315
34. Kos CH, Karaplis AC, Peng JB, Hediger MA, Goltzman D et al (2003) The calcium-sensing receptor is required for normal calcium homeostasis independent of parathyroid hormone. J Clin Invest 111: 1021–1028
35. Quarles LD (2003) Extracellular calcium-sensing receptors in the parathyroid gland, kidney, and other tissues. Curr Opin Nephrol Hypertens 12:349–355
36. Corbetta S, Mantovani G, Lania A, Borgato S, Vicentini L et al (2000) Calcium-sensing receptor expression and signalling in human parathyroid adenomas and primary hyperplasia. Clin Endocrinol (Oxf) 52:339–348
37. Levi R, Ben-Dov IZ, Lavi-Moshayoff V, Dinur M, Martin D et al (2006) Increased parathyroid hormone gene expression in secondary hyperparathyroidism of experimental uremia is reversed by calcimimetics: correlation with posttranslational modification of the trans acting factor AUF1. J Am Soc Nephrol 17:107–112
38. Colloton M, Shatzen E, Miller G, Stehman-Breen C, Wada M et al (2005) Cinacalcet HCl attenuates parathyroid hyperplasia in a rat model of secondary hyperparathyroidism. Kidney Int 67:467–476
39. Gupta SK, Khan TI, Gupta RC, Gupta AB, Gupta KC et al (2001) Compensatory hyperparathyroidism following high fluoride ingestion—a clinico-biochemical correlation. Indian Pediatr 38:139–146
40. Xu H, Liu QY, Zhang JM, Zhang H, Li GS (2010) Elevation of PTH and PTHrp induced by excessive fluoride in rats on a calcium-deficient diet. Biol Trace Elem Res 137:79–87
41. Park B, Yang YM, Choi BJ, Kim MS, Shin DM (2013) Activation of G proteins by aluminum fluoride enhances RANKL-mediated osteoclastogenesis. Korean J Physiol Pharmacol 17:427–433
42. von Tirpitz C, Klaus J, Steinkamp M, Hofbauer LC, Kratzer W et al (2003) Therapy of osteoporosis in patients with Crohn's disease: a randomized study comparing sodium fluoride and ibandronate. Aliment Pharmacol Ther 17:807–816