EFFECT OF FLUORIDE ON HUMAN DENTAL PULP CELLS *IN VITRO*

Sroisiri Thaweboon[1], Boonyanit Thaweboon[1], Panjit Chunhabundit[2] and Prapan Suppukpatana[2]

[1]Department of Microbiology, [2]Department of Anatomy, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Abstract. Human dental pulp cells were cultured in fluoride containing medium of various concentrations (0, 1, 2, 5, 10, 20, 25, 30, 40, 50, 60 and 80 ppm) in order to study the biological effect on the cells' proliferation and alkaline phosphatase (ALP) activities. It was found that fluoride at 5 ppm concentration significantly stimulated cell proliferation and ALP activity between 24 and 48 hours after exposure whereas at higher concentrations (40 - 80 ppm), fluoride significantly inhibited cell growth and ALP activity after 48 hours (Student's *t* test). The maximum effect was around 80 ppm. These observations suggest that fluoride, if used at a low concentration, may be a useful therapeutic agent for the treatment of pulpal disease by means of stimulating the proliferation and differentiation of dental pulp cells. At higher concentrations, it will have negative effects on this kind of cell.

INTRODUCTION

Sodium fluoride (NaF) has been used for decades, either systemically or topically, to prevent dental caries. Its positive effect against tooth decay has been well documented by numerous epidemiological studies, but ingestion of excess fluoride (F) results in fluorosis with clinically observed mottled enamel. Histological features of fluorosed enamel have shown structural changes with hypomineralized zones (Yanagisawa *et al*, 1989). The mechanisms by which an excess of F induces these enamel defects are not clear and may implicate a change in the synthesis rate or in the composition of secretory enamel proteins or the direct effect on the mineralization process (Richard *et al*, 1986). Dentinogenesis is also disturbed by high levels of F. Histological sections of fluorosed teeth have revealed an increased thickness of the predentin and some demineralized zones in the dentin.

Clinical studies of F in patients with osteoporosis have demonstrated that F increases spinal bone density (Riggs *et al*, 1980). Past bone histomorphometric studies have indicated that the effect of F to increase bone mass was due to an increase in bone formation and not to a reduction in bone resorption. This stimulation of bone formation was mediated through an increase in osteoblast proliferation (Briancon and Meunier, 1981; Harrison *et al*, 1981).

Dental pulp cells are primarily fibroblastic but are capable of differentiating into odontoblasts which form dentin in response to the injury. These cells have a number of features that are similar to those of osteoblastic cells (*ie* the presence of high alkaline phosphatase activity and the formation of calcified nodules in long-term cultures) (Nakanishi, 1991; Nakashima *et al*, 1994; Kasugai *et al*, 1993). In addition, dental pulp tissues autografted to non-pulpal sites can elaborate bone tissue (Yamamura, 1985), suggesting that pulp contains osteoprogenitor cells. Though there are biological and biochemical similarities between pulp cells and osteoblasts, studies on the effects of F on dental pulp have not been adequately investigated. The present study was designed to clarify the effects of high and low concentrations of F on the proliferation and alkaline phosphatase activity of normal human dental pulp cells *in vitro*.

Correspondence: Sroisiri Thaweboon, Department of Microbiology, Faculty of Dentistry, Mahidol University, 6 Yothi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2644 8644 ext 4811-2; Fax: 66 (0) 2644 8634
E-mail: dtstw@mahidol.ac.th

MATERIALS AND METHODS

Cell culture

Human dental pulp cells (DP-1 and DP-2) were obtained from teeth extracted from young patients (age 17 and 20 years) in the course of orth-

odontic treatment. After the dental pulps were removed under sterile conditions they were washed twice with phosphate buffer saline solution (PBS), pH 7.4. The pulpal tissues were minced and then placed in 35 mm tissue-culture dishes.

The culture medium used was Dulbecco's Modified Eagle medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), L-glutamine (Gibco), and antibiotics comprising 10,000 units of penicillin G (Gibco) and 25 µg/ml of amphotericin B (Gibco) as fungisone. Cultures were grown at 37°C in humidified atmosphere of 5% CO_2. After growing from the explant and confluencing, the cells were harvested with 0.05% trypsin (Gibco) in PBS and subcultured weekly. Cells from the 3rd to 6th passages were used.

Fluoride treatment

NaF was added by dilution of a 100-ppm stock solution with serum-free culture medium in order to obtain the following concentrations; 1, 2, 5, 10, 20, 25, 30, 40, 50, 60 and 80 ppm.

Cell growth

Cells were seeded in 96 microwell plates at a density of 10^4 cells/well. After 20 hours preincubation, F was added and the cell number was determined from a trypsinized cell suspension at 24 and 48 hours using tryphan blue and hemocytometer.

Alkaline phosphatase activity

Cells were seeded in 96 microwell plates at a density of 10^4 cells/well. After 20 hours preincubation, F was added and the cells were allowed to incubated for 48 hours. The alkaline phosphatase activity was determined using *p*-nitrophenylphosphate as substrate, according to Lowry *et al* (1951).

All results are shown as mean ±SD (n = 4). The statistical significance of the differences from the controls was determined with the Student's *t*-test. Any difference was considered significant when $p < 0.05$.

RESULTS

Cell growth

As shown in Figs 1 and 2, at 24 and 48 hours, F at 5 ppm significantly stimulated cell proliferation in both DP-1 and DP-2. The magnitude of the stimulation at 24 hours was 187.77% and 138.09%, at 48 hours was 144.44% and 140.00% for DP-1 and DP-2 respectively. Cell growth was significantly inhibited by F at 40 ppm or more after 48 hours for both cell strains.

Alkaline phosphatase activity

Fig 3 reveals a significant increase in the alkaline phosphatase activity in DP-1 and DP-2 at 5 ppm F, by 165.15% and 145.10% respectively. At high concentration (≥ 20 ppm), reduction in this activity was observed.

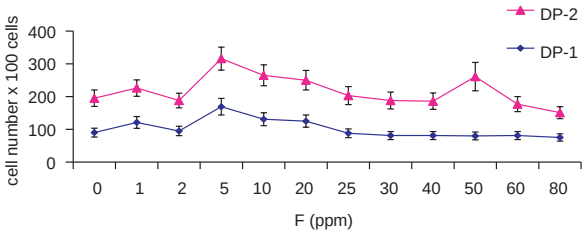

Fig 1–Effect of fluoride on the proliferation of human dental pulp cells at 24 hours. Results reported as mean ± SD (n = 4).

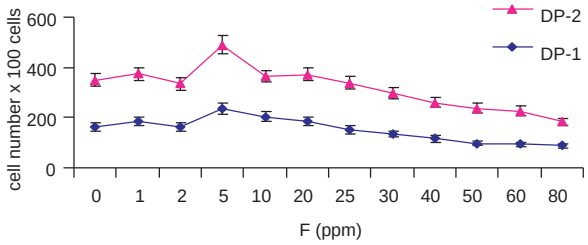

Fig 2–Effect of fluoride on the proliferation of human dental pulp cells at 48 hours. Results reported as mean ± SD (n = 4).

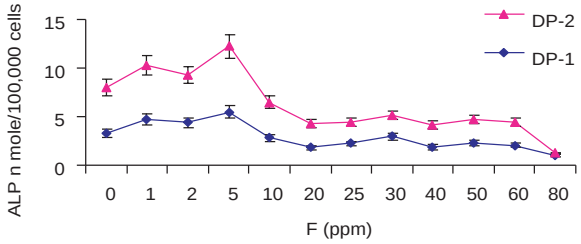

Fig 3–Effect of fluoride on alkaline phosphatase activity of human dental pulp cells at 48 hours. Results reported as mean ± SD (n = 4).

DISCUSSION

Fluoride was earlier shown to stimulate cell proliferation in monolayer cultures of osteoblastic cells, but not in parallel cultures of cells prepared from skin, muscle, liver, intestine, or kidney (Farley *et al*, 1988). It was also mitogenic for Green monkey kidney cells (Oguro *et al*, 1989). It therefore appears that the ability of fluoride to induce cell proliferation is tissue-specific. Nakade *et al* (1995) demonstrated that F at micromolar concentrations (2-4 ppm) significantly increased cell proliferation and alkaline phosphatase activity in normal human pulp cells. Our observations are in agreement with these data. The mitogenic activity of F to stimulate cell proliferation was first reported on bone cells by Farley *et al* (1983) with the optimal dose of micromolar levels. This bone cell effect of F was confirmed by a number of laboratories (Bellows *et al*, 1993; Burgener *et al*, 1995). There is evidence that F at mitogenic, micromolar doses also stimulates several mature osteoblast activities, *ie*, alkaline phosphatase expression, collagen synthesis, and osteocalcin synthesis in monolayer bone cell cultures (Lau and Baylink, 1998). The molecular mechanism of the mitogenic action of F is its involving the increases in overall tyrosine phosphorylation of several cellular signaling proteins, including mitogen activated protein kinase (MAPK) (Burgener *et al*, 1995; Wu *et al*, 1997). At high concentrations (≥ 25 ppm), F was found to inhibit cell growth and decrease alkaline phosphatase activity in human dental pulp cells (Veron *et al*, 1993). The inhibitory effect was suggested to be by blocking the normal cellular metabolism, inhibiting enzymes, in particular metalloenzymes involved in essential processes and interfering with necessary biochemical mechanisms controlled by calcium or other metal ions. The result of our present finding is rather different from that of Veron due to the inhibitory concentration of F. The responsiveness to F in Veron's study was determined in a medium containing 2% fetal calf serum, whereas our cultures contained no serum. In many studies about the F effect on cells, they were usually examined in the absence of serum (Lau *et al*, 1991; Wergedal *et al*, 1998). The effect of F on cells may be influenced by other variables such as the phosphate concentration in the medium and the availability of endogenous growth factor (Farley *et al*, 1983; 1988). Thus, the difference between our results and those of Veron might be due to the use of a different culture system.

Alkaline phosphatase activity is shown to be expressed in pulp cells and in odontoblasts (Linde and Granstrom, 1982), and thought to play a role in extracellular matrix mineralization processes (Wuthier and Register, 1985). It has been used as a valuable marker enzyme of odontoblast-like cells *in vitro* (Nakanishi, 1991), as well as bone cells (Wlodarski and Reddi, 1986). Our cultured cells expressed alkaline phosphatase activity that was stimulated in the presence of low F concentrations and inhibited by elevated concentrations. NaF is widely used in the treatment of osteoporosis and one of the responses to F treatment is an increase in serum alkaline phosphatase (Farley *et al*, 1987; Gruber and Baylink, 1991). Wergedal *et al* (1988) demonstrated that low concentrations of F induced an increased alkaline phosphatase level in exposed human bone cells. However, F effects on alkaline phosphatase level have varied according to the F concentrations used (Drapeau and Deshaies, 1983).

In conclusion, F at low concentrations can stimulate the proliferation and differentiated functions of human dental pulp cells whereas an inhibitory effect can be observed at higher concentrations. These data suggest that F, if used at low concentrations, could be a useful therapeutic agent where increased regeneration of dentin is desired.

REFERENCES

Bellows CG, Aubin JE, Heersche JN. Differential effects of fluoride during initiation and progression of mineralization of osteoid nodules formed *in vitro*. *J Bone Miner* 1993; 8: 1357-63.

Briancon D, Meunier PJ. Treatment of osteoporosis with fluoride, calcium and vitamin D.*Orthop Clin North Am* 1981; 12: 629-48.

Burgener D, Bonjour JP, Caverzasio J. Fluoride increases tyrosine kinase activity in osteoblast-like cells: regulatory role for the stimulation of cell proliferation and Pi transport across the plasma membrane. *J Bone Miner Res* 1995; 10: 164-71.

Drapeau AJ, Deshaies Y. Implication biologique du fluor diverse enzymes animales et végétales. *Ann Biol T*

XXVI, Fasc 1983; 3: 11-8.

Farley JR, Wergedal JE, Baylink DJ. Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone-forming cells. *Science* 1983; 222: 330-2.

Farley SM, Wergedal JE, Smith LC, Lundy MW, Farley JR, Baylink DJ. Fluoride therapy of osteoporosis: characterization of the skeletal response by serial measurements of serum alkaline phosphatase activity. *Metabolism* 1987; 36: 211.

Farley JR, Tarbaux N, Hall S, Baylink DJ. Evidence that fluoride stimulated [3H] thymidine incorporation in embryonic chick calvarial cell cultures; dependent on the presence of a bone cell mitogen, sensitive to changes in the phosphate concentration, and modulated by systemic skeletal effectors. *Metabolism* 1988; 37: 988-95.

Gruber HE, Baylink DJ. The effects of fluoride on bone. *Clin Orthop* 1991; 267: 264-77.

Harrison JE, McNeill KG, Sturtridge WC, *et al.* Three-year changes in bone mineral mass of osteoporotic patients based on neutron activation analysis of the central third of the skeleton. *J Clin Endocrinol Metab* 1981; 52: 751-8.

Kasugai S, Shibata S, Suzuki S, Suma T, Ogura H. Characterization of a system of mineralized-tissue formation by rat dentine pulp cells in culture. *Arch Oral Biol* 1993; 38: 769-71.

Lau KH, Baylink DJ. Molecular of action of fluoride on bone cells. *J Bone Miner Res* 1998; 13: 1660-7.

Lau KH, Yoo A, Wang SP. Aluminum stimulates the proliferation and differentiation of osteoblasts *in vitro* by a mechanism that is different from fluoride. *Mol Cell Biochem* 1991; 105: 93-105.

Linde A, Granstrom G. Odontoblast alkaline phosphatase and Ca++ transport. *J Biol Buccale* 1982; 6: 293-308.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.

Nakade O, Baylink DJ, Lau KH. Phenytoin at micromolar concentrations is an osteogenic agent for human-mandible-derived bone cells *in vitro*. *J Dent Res* 1995; 74: 331-7.

Nakanishi M. Establishment of primary cultures of pulp cells bovine permanent incisors. *Arch Oral Biol* 1991; 36: 655-63.

Nakashima M, Nagasawa H, Yamada Y, Raddi AH. Regulatory role of transforming growth factor-β bone morphogenetic protein-2, and protein-4 on gene expression of extracellular matrix proteins and differentiation of dental pulp cells. *Development Biol* 1994; 162: 18-22.

Oguro A, Koizumi H, Horii K. Effect of fluoride ion on proliferation of Vero cell line cells: growth acceleration by sodium fluoride. *J Dent Health* 1989; 31: 55-62.

Richard A, Kragstrup J, Josephen K, Fejerskov O. Dental fluorosis developed in post-secretory enamel. *J Dent Res* 1986; 65: 1406-9.

Riggs BL, Hodgson SF, Hoffman DL, Kelly PJ, Johnson KA, Taves D. Treatment of primary osteoporosis with fluoride and calcium: clinical tolerance and feature occurrence. *JAMA* 1980; 243: 446-9.

Veron MH, Couble ML, Magloire H. Selective inhibition of collagen synthesis by fluoride in human pulp fibroblasts *in vitro*. *Calcif Tissue Int* 1993; 53: 38-44.

Wergedal JE, Lau KHW, Baylink DL. Fluoride and bovine bone extract influence cell proliferation and phosphatase activities in human bone cell cultures. *Clin Orthop* 1988; 233: 274-82.

Wlodarski KH, Reddi AH. Alkaline phosphatase as a marker of osteoinductive cells. *Calcif Tissue Int* 1986; 39: 382-5.

Wu LW, Yoon HK, Baylink DJ, Graves LM, Lau KH. Fluoride at mitogenic doses induces a sustained activation of $p44^{mapk}$, but not $p42^{mapk}$, in human TE85 osteosarcoma cells. *J Clin Endocrinol Metab* 1997; 82: 1126-35.

Wuthier RE, Register TC. Role of alkaline phosphatase, a polyfunctional enzyme, in mineralizing tissues. In: Butler WT, ed. The chemistry and biology of mineralized tissues. Birmingham: Ebsco Media 1985: 113-24.

Yanagisawa Y, Takuma S, Fejuskov O. Ultrastructure and composition of enamel in human dental fluorosis. *Adv Dent Res* 1989; 3: 203-10.

Yamamura T. Differentiation of pulpal cells and inductive influences of various matrices with reference to pulp wound healing. *J Dent Res* 1985; 64: 530-40.