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). Dentino-genesis 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.

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.

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 ortho-
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% CO₂. 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 10⁴ 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 10⁴ 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.
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 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


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


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.


