DNA DAMAGE INDUCED BY FLUORIDE IN RAT OSTEOBLASTS

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SUMMARY: A study is reported of DNA damage by fluoride to primary calvarial osteoblasts of newborn rats isolated by enzymic digestion. Sodium fluoride at concentrations of 0, 0.5, 1, 2, and 3 mmol/L was administered to the isolated osteoblast cells for 24 hr, and damage to DNA was determined by single cell gel electrophoresis assay (SCGE = Comet assay). Breakage of DNA strands occurred at 2 mmol /L NaF and above. Compared with the control group, the comet tail length was significantly increased, indicating that, at sufficient concentrations, fluoride can induce DNA damage in osteoblasts.

Keywords: Comet assay; DNA damage; Rat osteoblasts; Single cell gel electrophoresis; Sodium fluoride.

INTRODUCTION

Although fluoride (F) has been used in the treatment of osteoporosis because it is known to increase bone mass, the quality of bone formed is poor, and excessive intake causes skeletal fluorosis. Moreover, there is some controversy about its genotoxic effects. For example, Ru-qing Xu[1] reported that chromosomal aberrations and DNA damage can be detected before the X-ray images of skeletal fluorosis appear in F-exposed people. Likewise, Jie Li et al.[2] found that the peripheral lymphocytes of patients afflicted with skeletal fluorosis had high frequencies of micronuclei and sister-chromatid exchanges, thereby indicating that high F concentration can result in both double and single DNA strand breaks in cells. However, other studies have not found genotoxic effects of F and have only shown that it can affect cellular functions and enzyme activities.[3,4]

Single-cell gel electrophoresis assay, also known as the "comet" assay, is a fairly new, rapid, simple, and reliable biochemical technique for evaluating DNA damage in mammalian cells.[5] In this study, comet assay was used to analyze DNA damage in osteoblasts isolated from newborn rat bones following exposure of the cell cultures to various concentrations of F.

MATERIALS AND METHODS

Materials: Two-day-old Wistar rats were provided by the Department of Experimental Animals, China Medical University. The 199 culture medium was supplied by Gibco and sodium fluoride (NaF) by the Chemical Plant of Beijing. Collagenase II was obtained from Sigma and the collagen I immunohistochemistry kit from the Boston Company, Wuhan, China. Trypsin, normal melting temperature agarose (NMA), low melting temperature agarose (LMA), Triton X-100, and ethidium bromide (EB) were all obtained from the Sino American Biotechnology Co.

Cell Culture: Calvariae from ten 2-day-old newborn rats were removed aseptically. The periosteal layers on both sides were carefully stripped off with tweezers under D-Hanks solution. The bone specimens were trypsinized with

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0.25% trypsin, and the specimens were digested by collagenase containing 1 mg collagenase II/mL. The supernatant was then collected, centrifuged, and purified by the differential adherence method. The released cells were adjusted to 10^6/mL and cultured in 25-mL tissue culture flasks (Gibco) plated with 199 medium supplemented with 15% fetal bovine serum (FBS). After two days the culture medium was replaced, and the osteoblasts obtained in this way were shown to express type I collagen by immunohistochemical testing.

Detecting DNA damage: When the cells in each flask had been grown to confluence, the medium was changed to one that was serum-free. After 24 hr, the cells were exposed to 0, 0.5, 1, 2, 3 mmol/L NaF for a further 24 hr. The cells were then trypsinized, washed twice in PBS, and the cell number was adjusted to a density of 1×10^6 cells/mL. Comet assay by the method of Singh et al.[5] was used to study the DNA damage. In brief, the steps were: Preservation of comet assay slides — Lysis of cells — Unfolding — Electrophoresis — Neutralization — Staining — Observation.

Observations were made using a fluorescence microscope. The lengths of 100 cells in every slide were measured randomly with an ocular micrometer.

Statistics: At least three experiments were performed for each experimental condition. Results are expressed as mean ± SD. Statistical differences were analyzed using ANOVA by SPSS 10.0.

RESULTS

Cell isolation: After isolating and culturing for 24 h, the active osteoblasts adhered to the walls of the flasks, and showed a spindly, triangular, or polyangular appearance.

Positive results of immunohistochemical testing are defined as brown granules distributed in the cytoplasm of osteoblasts, which are type I collagen secreted by osteoblasts with slightly stained nuclei.

Cytotoxicity of NaF to osteoblasts: Cell survivals of various concentrations of NaF were all above 80% except in the 3 mmol/L group, which was 60%.

The DNA damage induced by NaF: Under the same cell culture conditions, there were different levels of DNA strand breakage after exposure to NaF from 0–3 mmol/L. At 0.5–1 mmol NaF/L, the DNA tail length increased, but the increase was not statistically significant compared with the control. At 2–3 nmol NaF/L, breakage of DNA strands in the osteoblasts occurred, and the DNA tail length was significantly increased (Table below). The migration distance of DNA at 3 mmol NaF/L was also significant compared to 2 mmol/L.

Table. DNA damage by comet assay in cultured rat osteoblasts induced by NaF

NaF (mmol/L)	Cell number	Tail length (nm ±SD)
0 (control)	100	6.390 ± 5.927
0.5	100	7.080 ± 6.068
2	100	7.620 ± 5.486
2	100	10.770 ± 5.581*
3	100	14.930 ± 11.754*

*Values differ significantly from the control: $p<0.05$.

DISCUSSION

Excessive intake of F via drinking water and other sources is an endemic problem in a number of countries, including, among others, China, India, and Mexico.[6] It is thought by some researchers that F can induce changes in nucleic acids. Investigating the effects of F on the DNA and RNA content of heart, liver, and kidney of fluorosed rats, one group has found that high concentrations of NaF inhibited the synthesis of DNA and RNA in cultured cells of these organs and reduced the content of RNA in them.[7] Other studies suggest that F can also induce DNA damage. Anismov and Bolotnikov[9] stained K562 cells with trypan blue after treatment with AlF_4^- and found that DNA fragmentation increased 2- to 4-fold when thymine was labeled with tritium. He and Chen[10] gave Sprague-Dawley rats 150 mg NaF/L in the drinking water and after 4 weeks found that F had induced oxidative stress and DNA damage, leading to apoptosis and cell cycle changes in rat oral mucosal cells and hepatocytes.[10]

In the present study we used the comet assay to investigate the genotoxic potential of F to newborn rat osteoblasts. The results showed that, under otherwise identical conditions, different concentrations of NaF could induce different degrees of DNA damage. The migration of DNA (comet tail) increased compared with the controls when using 0.5–1 mmol NaF/L, but the increase was not statistically significant, thus suggesting no appreciable cell damage. However, at 2 mmol NaF/L, DNA strand breaks in osteoblasts occurred, even though the cell survival rate was 85%. We conclude therefore that long-term action of the higher F concentration could induce DNA damage to osteoblasts, although the genetic toxicity of F is weaker than other genetic toxicants.

We also found that at 3 mmol NaF/L survival of cells was reduced to 60% after 24 hr of treatment. It is known that “comet” cells may occur owing to the death of cells but without genetic intoxication, thus giving a false-positive message. Consequently, it is necessary to determine the cytotoxicity of toxicants in comet assay,[11] and the survival of cells should be above 80% in the detection of genetic toxicity.

In conclusion, DNA strand breaks in osteoblasts were found to occur when rat osteoblasts were exposed to NaF at concentrations of 2 mmol/L and above. These findings demonstrate that certain concentrations of F can damage DNA in osteoblasts, and excessive intake and accumulation of F is therefore a serious risk factor for adverse development of bone.

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