DNA DAMAGE INDUCED BY FLUORIDE IN RAT KIDNEY CELLS

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SUMMARY: DNA damage by fluoride to newborn rat kidney cells isolated by enzymic digestion is reported. The cells were exposed for 24 hr to sodium fluoride at NaF concentrations of 0, 0.2, 0.4, 0.8, and 1.0 mM. Damage to DNA was determined by single cell gel electrophoresis assay (Comet assay). Significant breakage of DNA strands occurred at 0.8 mM 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 kidney cells.

Keywords: DNA damage; Rat kidney cells; Single cell gel electrophoresis; Sodium fluoride.

INTRODUCTION

Endemic fluorosis, which often seriously impairs human health, is prevalent in many parts of the world. Fluorosis causes damage not only to skeletal tissue and teeth, but also to soft tissues, such as brain, liver, kidney, and spinal cord. Some studies indicate that fluoride (F) can cause DNA damaged and chromosome aberrations.[1-2] However, other studies have not found genotoxic effects of F. [3-4]

Single-cell gel electrophoresis assay, also known as the "comet" assay, is a fairly recent, rapid, simple, and reliable biochemical technique for evaluating DNA damage in mammalian cells.[5] Since about fifty percent of daily F intake is excreted through the kidneys, this study, making use of the comet assay, was undertaken to analyze DNA damage in kidney cells isolated from newborn rats following exposure of the cell cultures to various increasing concentrations of F as NaF.

MATERIALS AND METHODS

Materials: Two-day-old male Wistar rats were provided by the Department of Experimental Animals, China Medical University. The RPMI1640 culture medium and collagenase IV were supplied by Gibco and sodium fluoride (NaF) by the Chemical Plant of Beijing. 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: Kidneys from four two-day-old newborn rats were removed aseptically. The renal capsules were firstly removed with nipper and scissors, and then the kidneys were sliced longitudinally. The renal cortex was dissected from the medulla, sliced, and minced in D-Hanks solution. The small cubes of renal cortex tissue were washed three times by D-Hanks solution and the supernatant was discarded, the deposition was digested by collagenase containing 0.05% collagenase IV at 37ºC. The supernatant was then collected and passed through a 250-micron filter aseptically in order to remove remnant tissue. The cell suspension was then centrifuged at 800 r/min for 5 min. The resulting pellets were resuspended in RPMI 1640 with 10% fetal bovine serum. The released cells were

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adjusted to 10^6/mL and cultured in 100 cm^2 tissue culture flasks (Gibco) plated with RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cell cultures were maintained at 37ºC in a humidified atmosphere of 95% air and 5% CO_2. After one day the culture medium was replaced, and when the cells in each flask had grown to confluence, they were subcultured with 0.25% trypsin according to the common method of cell passaging. Finally, the cells were divided into two portions—one for the plates and the other for the culture flasks.

MTT assay for cell viability: When the cells in the 96 plates had grown to confluence, the medium was changed to one that was serum-free. After 24 hr, the cells were exposed to 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mM NaF for a further 24 hr. The MTT reduction assay was used as a qualitative index of cell viability. The optical densities were read at 555 nm using a microplate spectrophotometer (Bio-Tek Instruments. Inc. USA). The absorbance values for controls averaged from 1.8 to 2.1 OD units. Control values were calculated as the mean of 6 absorbance values; each control was tested in triplicate in two separate experiments. We calculated cytotoxicity by expressing experimental absorbance values as percentage of control values.

Detecting DNA damage: When the cells in each flask had grown to confluence, the medium was changed to one that was serum-free. After 24 hr, the cells were exposed to 0, 0.2, 0.4, 0.8, and 1.0 mM 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 and 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 sets of experiments were performed for each experimental condition. Results are expressed as mean ± SD. Statistical differences were analyzed using ANOVA by SPSS 10.0.

RESULTS

Cytotoxicity of NaF to isolated kidney cells: The viability of the kidney cells exposed to increasing concentrations of NaF for 24 hr is shown in the figure, in which it appears that the viability of the cells was noticeably increased at 0.2 mM NaF (to 127.5%) and was significantly decreased at 0.8 and 1.0 mM (to 81.6 and 70.9%, respectively). At 0.4 and 0.6 mM NaF there was little effect on cell viability.

DNA damage induced by NaF: Under the same cell culture conditions, different degrees of DNA strand breakage in the presence of NaF. At 0.2 and 0.4 mM NaF, the DNA tail length increased, but the increase was not statistically significant compared with the control. At 0.8 and 1.0 mM NaF, breakage of DNA strands in

the kidney cells occurred, and the DNA tail length was significantly increased (see Table).

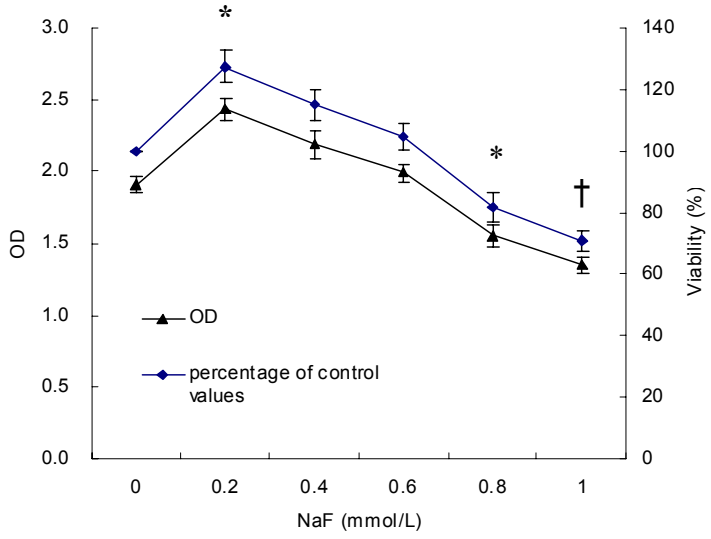

Figure. The effects of NaF on cell viability measured by the MTT assay. The rat kidney cells viability with 24 hr NaF exposure. Values are mean ± SD of data from two separate experiments; each experiment was performed in triplicate. *p <0.05, †p <0.01 versus control.

Table. DNA damage induced by NaF as detected by comet assay in cultured rat kidney cells

NaF (mM)	Cell number	Tail length (nm±SD)
0 (control)	100	4.267±2.172
0.2	100	5.410±3.061
0.4	100	5.170±2.571
0.8	100	8.970±3.106*
1.0	100	10.631±5.724†

*P<0.05, †P<0.01 versus control.

DISCUSSION

Damage to kidneys by F manifests itself by epithelial cell swelling, hydropic degeneration of all proximal and distal tubular segments, and the appearance of intraluminal casts.[6] NaF has been shown to induce apoptosis in renal tubules via activation of the Bax expression and Bcl-2 suppression.[7] It has also been found to decrease urinary alpha-glutathione-S-transferase, which is useful as marker of S3 proximal tubule damage.[8] These results suggest that the toxic effect of F on the kidney may be more pronounced in the proximal tubule than in the glomeruli region. Because the renal cortex consists mainly of tubular epithelial cells, we chose these cells for study instead of all of the kidney cells.

For our study we used the comet assay to investigate the genotoxic potential of F to the kidney cell cultures derived from newborn rats. The results showed that, under otherwise identical conditions, different concentrations of NaF induced different degrees of DNA damage. The migration of DNA (comet tail) increased compared with the controls in the presence of 0.2 and 0.4 mM NaF, but the increase was not statistically significant. However, beginning at 0.8 mM NaF,

DNA strand breaks occurred, even though the cell survival rate was 81.6%. We conclude, therefore, that long-term action of F at high enough concentrations can induce DNA damage to kidney cells. Recently Błaszczyk et al. confirmed the deleterious effect of NaF on the antioxidative system in rat kidney (increase in the concentration MDA, decreased activity of all antioxidative enzymes).[9] Consequently, it is value to conduct further study to see if F induces DNA damage in kidney cells by oxidative stress.

As expected and seen in the Figure, the viability of the cells decreased at the higher 0.8 and 1.0 mM NaF concentrations compared to the control level. However, the viability actually increased at 0.2 mM NaF, thereby indicating a stimulatory or hormesis effect at low F concentration. Thus, while F clearly inhibited cell proliferation at higher concentrations, it also stimulated cell proliferation at low concentration, creating a paradoxical concentration effect.[10] Since the viability of the cells was reduced below 80% at 1.0 mM NaF, a false-positive message from the comet assay at this concentration cannot be excluded.[11]

In conclusion, DNA strand breaks were found to occur *in vitro* in rat kidney cells at NaF at concentrations of 0.8 mM and above over a period of 24 hr. On the other hand, significant breakage of DNA strands in rat osteoblasts was not observed until the NaF concentration reached 2.0 mM.[12] Thus excessive F intake appears to be a more serious risk factor for injury to soft tissue cells like those of the kidney than to hard tissue bone cells.

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