Analysis of Chromosomal Abnormalities at Anaphase-Telophase Induced by Sodium Fluoride *In Vitro*

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In recent years, discharge of industrial wastes and the application of chemicals to the natural environment have caused air, water, and soil pollution. The contamination with these chemicals has created potential hazards as mutagens, teratogens, and/or carcinogens to both plants and animals life, including humans.

Fluorocompounds are potential air pollutants in both rural and urban areas. Earlier studies on the cytogenetic effects of fluoride have been conducted primarily in plants. Sodium fluoride in concentration of 1×10^{-6} M or higher was shown to induce chromosomal abnormalities in onion (Mohamed *et al.* 1966a), tomato plants (Mohamed *et al.* 1966b), maize (Mohamed 1970), and barley (Bale and Hart 1973a, b). Jachimczak and Skotarczak (1978) showed that fluoride causes chromosomal abnormalities in cultured leucocytes. *In vitro* treatment of oocytes from mice, ewes, and cows with sodium fluoride induced chromosomal abnormalities (Jagiello and Lin 1974). On the other hand some investigators have pointed out that fluoride at low concentration failed to produce chromosomal anomalies (Kram *et al.* 1978, Leonard *et al.* 1977, Obe and Slacik-Erben 1973).

Sodium fluoride has been reported to induce morphological and neoplastic transformation, chromosomal abnormalities, sister chromatid exchanges, and unscheduled DNA synthesis (Tsutsui *et al.* 1984a, b, c).

The primary objective of this study was to attempt to observe any effects of fluoride on Don Chinese hamster cells. This objective was approached by analyzing different types of chromosomal abnormalities encountered after treatment of cells with various concentrations of fluoride.

Materials and methods

Chemicals

Technical grade sodium fluoride was purchased from Fisher Scientific Company. Alpha minimum essential medium (Alpha MEM), fetal bovine serum, and trypsin EDTA were purchased from K. C. Biological Company. Gentamycin was purchased from Schreing Corporation.

Anaphase-Telophase Test

Don Chinese hamster cells were obtained from the American Type Culture Collection. The cells were grown in 25 cm² disposable plastic flasks in a carbon dioxide incubator at 37°C.

Alpha MEM supplemented with 10% fetal bovine serum and 50 $\mu\text{g/ml}$ gentamycin was used for experimental cultures.

Figs. 1-12. Types of chromosomal abnormalities induced by sodium fluoride in Don Chinese hamster cells. 1, bridge. 2, double bridge. 3, sidearm bridge. 4, bridge with fragments. 5, tripolar anaphase with bridge. 6, multipolar anaphase with bridge. 7, tripolar anaphase. 8, multipolar anaphase. 9, fragments. 10, laggard. 11, 'Y' metaphase. 12, 'X' metaphase.

For Anaphase-Telophase tests, approximately 1×10^5 cells were seeded in Tissue-tek flaskettes and allowed to grow for 12 hours. The flaskettes were replaced with fresh medium containing 0 μg , 25 μg , 50 μg , and 75 $\mu\text{g}/\text{ml}$ of sodium fluoride (NaF). At the end of each treatment period, the cells were washed with phosphate buffered saline and fixed with Carnoy's solution (1 part glacial acetic acid and 3 parts of methanol). The slides were separated from the flaskettes and the cells were flame fixed. They were stained with acetocarmine or aceto-

Table 1. Types and frequencies of chromosomal abnormalities observed in Don Chinese hamster cells after treatment with sodium fluoride

Concentration and treatment period	Numbers of cells scored	Fragments	Bridges	Bridges + fragment	Sidearm bridge	Tripolar* anaphase	Multi-polar* anaphase	Laggard	Total %
0 $\mu\text{g}/\text{ml}$	200	5	10	0	1	2	0	3	10.5
25 $\mu\text{g}/\text{ml}$	200	12	22	1	6	2	0	1	22.0
50 $\mu\text{g}/\text{ml}$	200	10	26	2	2	4	1	3	24.0
75 $\mu\text{g}/\text{ml}$	200	11	16	2	6	2	1	2	20.0
0 $\mu\text{g}/\text{ml}$	200	6	9	1	2	1	0	0	9.5
25 $\mu\text{g}/\text{ml}$	200	16	30	6	2	1	2	3	30.6
50 $\mu\text{g}/\text{ml}$	200	12	34	0	0	4	1	3	27.0
75 $\mu\text{g}/\text{ml}$	200	18	38	8	0	5	0	1	35.0
0 $\mu\text{g}/\text{ml}$	200	5	11	1	2	1	0	0	15.0
25 $\mu\text{g}/\text{ml}$	200	6	29	5	0	4	0	4	24.0
50 $\mu\text{g}/\text{ml}$	200	7	39	2	0	0	2	3	26.0
75 $\mu\text{g}/\text{ml}$	200	7	35	4	3	3	1	1	27.0

* With or without bridge.

Table 2. Analysis of variance for the total chromosomal aberrations induced by sodium fluoride in Don Chinese hamster cells

Source of variation	Degrees of freedom	Sum square	Mean square	F
Total	11	649.23		
Treatments	3	479.56	159.85	8.52*
Periods	2	57.11	28.53	1.52
Error	6	112.56	18.76	

* Significant at 1 and 5% probability level.

Table 3. Duncan Multiple Range Test for the total chromosomal abnormalities induced by sodium fluoride in Don Chinese hamster cells

Concentration	0 $\mu\text{g}/\text{ml}$	25 $\mu\text{g}/\text{ml}$	50 $\mu\text{g}/\text{ml}$	75 $\mu\text{g}/\text{ml}$
Mean*	11.66	25.33	25.83	27.33

* Values connected by the same line do not differ significantly at the 5% probability level.

orcein and made permanent. Chromosomal abnormalities were scored using late anaphase and early telophase stages.

Results

Chromosomal abnormalities induced by NaF in Don Chinese hamster cells are presented in Table 1. NaF induced chromosomal aberrations in all the concentrations used. A 24 hour

period of treatment in all concentrations of fluoride produced maximum effect. Cytological observations made at anaphase and telophase stages revealed several kinds of abnormalities. The chief abnormalities observed were bridges, double bridges, sidearm bridges, bridges with fragments, tripolar and multipolar anaphases with or without bridges, fragments, and laggards (Figs. 1-10). In addition to these abnormalities 'Y' and 'X' configurations were also observed at metaphase (Figs. 11, 12). Bridges and fragments were common in most of the treatments.

A statistical analysis of the total chromosomal abnormalities observed following NaF treatment for varying periods showed that the mean square for the treatments was statistically significant. However, mean square for the periods was not significant (Table 2).

A Duncan's multiple range test was employed to compare the treatment means. The effect of fluoride at all levels used was found to be non-significant. However, the effects of 25 μg , 50 μg , and 75 $\mu\text{g}/\text{ml}$ of NaF were significant at the 5% probability level as compared to control (Table 3).

Discussion

It is clear that each of the concentrations of NaF is capable of inducing chromosomal abnormalities. It appears that a 24 hour period for all the levels of fluoride produced the maximum effect. The statistical analysis for chromosomal abnormalities revealed the results to highly significant.

Bridges scored in anaphase-telophase may be due to stickiness of chromosomes or due to exchange between chromosomes or chromatids (Davidson 1957). However, it is not clearly established whether naturally occurring and fluoride induced bridges are of the same type. Lagging chromosomes or fragments observed can originate from alterations of the mitotic spindle, or of the centromere, or from chromosome or chromatid breaks (Dulot and Olivero 1984). The fragments and laggards appeared as micronuclei at a later stage.

It has been shown that NaF affects the rate of protein synthesis (Chang 1970, Vesco and Colombo 1970). Since the mitotic spindle fibers are composed of proteins, it is apparent that fluoride could be responsible for the disruption of microtubules, causing 'Y' and 'X' type of configurations in metaphase. Such disorientation may produce tripolar and multipolar anaphases.

Although some investigators (Kram *et al.* 1978, Leonard *et al.* 1977, Obe and Slacik-Erben 1973) have indicated that fluoride is unable to produce chromosome abnormalities, the present study indicates that NaF can induce chromosomal changes in Don Chinese hamster cells. Similar observations were made in other mammalian cells by other investigators (Jachimaczak and Skotarczak 1978, Mohamed and Chandler 1982, Tsutsui *et al.* 1984a, b).

The foregoing observations have shown that exposure of Don cells to NaF induced a variety of chromosomal abnormalities and thus fluoride may be considered to be clastogenic.

Summary

The effect of treatment of cultured Don Chinese hamster cells with sodium fluoride has been investigated with respect to induction of chromosomal abnormalities. Exposure of Don Chinese hamster cells to sodium fluoride (25 $\mu\text{g}/\text{ml}$, 50 $\mu\text{g}/\text{ml}$, and 75 $\mu\text{g}/\text{ml}$) induced a higher frequency of chromosomal abnormalities as compared to control. The observed abnormalities included were bridges, bridges with fragments, sidearm bridges and tripolar and multipolar anaphases. Bridges and fragments were the most common type of abnormalities observed in all treatments.

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