SODIUM FLUORIDE INDUCED CHROMOSOME ABERRATIONS AND SISTER CHROMATID EXCHANGE IN CULTURED HUMAN LYMPHOCYTES

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SUMMARY: Experimental sodium fluoride (NaF) up to 30 times the level recommended in drinking water (1 ppm) was compared with an inorganic salt for its ability to induce chromosome aberrations and sister chromatid exchange (SCE) in cultured human lymphocytes. An increase in the frequencies of chromosome aberrations but not of SCE was found.

Key words: Chromosome aberration; Human lymphocytes; Sister chromatid exchange; Sodium fluoride.

INTRODUCTION

Fluoride is a ubiquitous substance found in food and water and extensively utilised for industrial purposes. Fluoride emissions have a direct or indirect effect upon living organisms and have been subjected to many genotoxicity experiments.

An early study on bean (*Vicia faba*) reported no fluoride-induced chromosomal aberrations.[1] On the other hand, A H Mohamed later reported positive results with tomato,[2,3] corn,[4] and onion[5]. In subsequent *in vivo* experiments Mohamed reported a significant increase in chromosome aberrations in mice fed on drinking water containing fluoride concentrations as low as 1.0 ppm,[6] but later apparently joined in rebutting such finding.[7]

It was observed that fluoride induces morphological and neoplastic transformations, chromosome aberrations, sister chromatid exchanges and unscheduled DNA synthesis in cultured Syrian hamster embryo (SHE) cells.[8] No increase in chromosome aberrations in human fibroblasts was reported following prolonged exposure to low concentrations of sodium fluoride.[9]

However, in cultured human lymphocytes sodium fluoride induced chromosomal aberration,[10] though Klein *et al* found no effect of fluoride on lymphocytes.[11] More recently, the significant increase in sister chromatid exchange (SCE) rate of persons exposed to fluoride in the endemic (1.98 to 2.2 ppm) area of North Gujarat has been reported.[12]

In view of such conflicting reports, it was decided to compare the *in vitro* effects of sodium fluoride and another inorganic salt upon the induction of chromosome aberrations and frequencies of SCE in cultured human lymphocytes.

MATERIALS AND METHODS

Peripheral blood samples were obtained from 50 individuals. Blood cultures set up each containing 0.6 mL of RPML 1640 supplemented with 20% fetal calf serum and 0.2 mL reconstituted PHA (Sigma). After 24-hours of incubation NaF (10, 20 and 30 μg/mL), NaCl (Chemically related control, concentration 10, 20 and 30 μg/mL) and Mytomycin C (Positive control, 25 and 50 ng/mL) were added to the cultures. Colcemid (0.5 μg/mL) added at 69-hours. After two hours the cultures were given hypotonic treatment (0.075M KCL). The cells

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TABLE 1. Chromosome aberration categories

Treatment	Duration	Dose per mL	No. of people sampled	No. of cells scored	Mean age	Aberrations/100 cells						Total aberration - gaps
						G	D	E	Dic	O	F	
Control	72 hrs	-	50	5000	32.6	42.5	4.5	0.0	0.0	0.0	0.0	4.5
NaCl	48 hrs	10 µg	50	4950		73.5	3.5	0.0	0.0	0.0	1.0	4.5
(NS)	(24-72 h)	20 µg	50	4910	32.6	77.0	5.0	0.0	0.0	0.0	0.0	6.0
		30 µg	50	4880		81.5	6.0	0.0	0.0	0.0	0.0	8.0
NaF	48 hrs	10 µg	50	4920		88.5	5.5	0.0	0.0	1.0	0.0	6.5
(NS)	(24-72 h)	20 µg	50	4930	32.6	92.0	5.0	0.0	0.0	2.0	1.0	8.0
		30 µg	50	4870		97.5	4.0	2.0	1.0	1.0	1.0	9.0
MMC *	48 hrs	25 ng	50	4810	32.6	78.0	93.5	43.0	1.0	3.0	5.0	145.5
	(24-72 h)	50ng	50	4790		95.5	115.0	55.0	3.0	5.0	9.0	187.0

G = Gaps, D = Deletion E = Exchange Dic = Dicentric O = Ring F = Fragments NS = Not Significant

* Significantly different from control ($p < 0.01$)

were fixed (3:1 methanol:acetic acid) and air-dried chromosome preparations was done. The slides were stained in 3% Giesma. For SCE metaphase chromosome preparations from blood samples of treatment and controls were carried out by routine phytohaemagglutinin (PHA) stimulated cultures. 5-Bromodeoxyuridine (BrdU) was added at final concentration of 10 μg/mL after 24 hours of stimulation. The fluorescence plus Giesma method was used for scoring sister chromatid exchanges.[13]

All slides were randomised with code numbers and were scanned by a single individual. A total of 100 first division metaphases from each culture was scored for chromosome aberrations and 25 second division metaphases were scored for SCE.

RESULTS

The frequencies of chromosome aberrations are presented in Table 1. The highest concentration (30 μg/mL) of sodium chloride (NaCl) showed higher frequencies of chromatid aberrations as compared to control, but these were mainly chromatid gaps. Similarly at the highest concentration (30 μg/mL) of sodium fluoride used in the present study also showed a slight elevation in the level of chromosome aberrations, over both the control and chemically related control, that includes mainly chromatid gaps one dicentric and one ring. The NaF induced treatment of gaps was similar to the MMC positive control.

The frequencies of sister SCEs after various treatments are shown in Table 2. Sodium fluoride and sodium chloride did not induce any significant increase in SCE level as compared to controls.

TABLE 2. Sister Chromatid exchange frequencies after various treatments

Treatment	Duration	Dose per mL	No. of People sampled	No. of cells scored	No. of metaphases with 2nd cell cycle	SCE/CELL
Control	72 hrs	-	50	1250	420	7.3 + 0.54
NaCl	48 hrs	10µg	50	1220	410	7.4 + 0.70
(NS)	(24-72 h)	20 µg	50	1205	395	7.5 + 0.60
		30 µg	50	1195	380	7.9 + 0.70
NaF	48 hrs	10µg	50	1210	405	7.7 + 0.60
(NS)	(24-72 h)	20 µg	50	1195	390	7.9 + 0.70
		30 µg	50	1180	390	7.9 + 0.70
MMC	48 hrs	25 ng	50	1150	320	24.5 ± 2.10*
	(24-72 h)	50 ng	50	1110	300	31.0 ± 3.00**

Significantly different from control: * $p < 0.01$ ** $p < 0.001$

DISCUSSION

Fluoride is generally admitted to cause chromosome damage *in vitro*. *In vivo* studies are where the controversy exists.[14] The conclusion derived from the report[8] that fluoride induces chromosome aberrations was based solely on the presence of chromatid gaps and breaks, which have been reported to be unreliable indicators of real damage to the genome.[15]

The concentration of sodium fluoride tested in the present study was 10, 20 and 30 μg/mL which was 10-30 times the level used in the fluoridation of drinking water (1 ppm). An increase in the frequencies of chromosome aberrations (whether gaps are excluded or not), but no appreciable increase of SCEs was noticed after treatment with sodium fluoride (Tables 1 and 2). As expected, exposure of culture to MMC proved to be clastogenic and carcinogenic and produced significant increase in chromosome aberrations and SCEs.

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