IN VIVO SUPPRESSION BY FLUORIDE OF CHROMOSOME ABERRATIONS INDUCED BY MITOMYCIN-C IN MOUSE BONE MARROW CELLS

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SUMMARY: *In vivo* clastogenic effects of mitomycin-C (MMC) in bone marrow cells of four groups of young male Swiss albino mice exposed to 0, 7.5, 15, and 30 mg NaF/L in their drinking water for 30 days were investigated. The percentages of aberrant metaphases and chromosome aberrations in all F-treated mice were significantly increased. In another experiment, NaF pretreatment was followed by intraperitoneal administration of 4 mg MMC/kg bw. Results indicated that the two chemicals did not have a synergistic effect; instead, a notable suppression of the clastogenic effect of MMC occurred. As expected, mitotic indices (MI) in the bone marrow cells of the MMC-treated mice were significantly lower than in the controls. However, in the mice pretreated with 30 mg NaF/L, the percent of MI was greater than in the MMC-treated group without NaF exposure. Moreover, the percentages of aberrant metaphases and chromatid breaks were significantly higher in all the F groups than in the controls. NaF exposure of the mice for 30 days evidently also helped prevent MMC-induced damage, although the effect was not statistically significant. In the mice pre-exposed to 30 mg NaF/L in their drinking water without treatment with MMC, a 10% decrease in chromatid breaks was observed. Thus these *in vivo* findings corroborate earlier reports of clastogenic effects of NaF treatment *in vitro*.

Keywords: Chromatid breaks; Chromosome aberrations; Mitomycin-C; Mitotic index; Mouse bone marrow cells.

INTRODUCTION

Contradictory findings, either *in vitro* or *in vivo* have been reported on the effect of NaF on alkylating agent-induced chromosome aberrations in human peripheral blood lymphocytes[1,2] and in the human population of endemic fluoride areas.[3] Mitomycin-C (MMC) is a bifunctional alkylating agent used as an anti-cancer chemotherapeutic agent. It binds covalently with DNA, delays the cell cycle, and produces chromosome aberrations. In the present study the *in vivo* effect of MMC was observed in bone marrow cells of mice that were or were not exposed to NaF in their drinking water.

MATERIALS AND METHODS

Male Swiss albino mice aged 2–3 months and weighing 25–30 g, were maintained in community cages in a controlled-temperature room (20±2ºC) and a 12-hr light/12-hr dark cycle. The animals were provided with standard mouse diet (NMC Oil Mills Ltd, Pune, India) and water *ad libitum.* All regulations of the Committee on Institutional Animal Care and Use were strictly followed during the experiment to protect the welfare of the animals. A total of 32 mice were kept in groups of eight and treated through their drinking water with 0, 7.5, 15, or 30 mg

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NaF/L for 30 days. For MMC treatment, a solution of MMC was prepared in a pH 7.4 phosphate buffer (PBS) and injected intraperitoneally at a level of 4 mg/kg bw. An equal volume of PBS was injected into the untreated control mice.

At the end of the 30-day period, the mice were killed by cervical dislocation. The femurs were dissected out, and the bone marrow cells were flushed out using sterile disposable syringe with 2 mL of 0.075M KCl hypotonic solution prewarmed to 37ºC. The cells were fixed at 13 hr to harvest a maximum number of cells in the first cycles.[4] Colchicine (10 mg/kg bw) was injected 3 hr before the harvesting to arrest cells at metaphases. Cells were kept in the hypotonic solution at 37ºC for 18 min; collected by centrifugation and fixed in acetic acid and methanol (1:3). Slides were prepared by the flame drying method and stained in 5% Giemsa for 5 min and mounted. For chromosome aberration studies, a minimum of 100 well spread metaphase plates were observed under a microscope with 100 × 10 magnification from randomly coded slides.

The results in the table were analyzed by two-tailed Student's t test by the Origin 6.1 statistical package.

RESULTS

As seen in the table, administration of 4 mg of mitomycin-C/kg bw significantly lowered the percentage of mitotic indices (MI) in all groups compared to the control group. The percentages of MI in the NaF-exposed groups, however, were not significantly different from those of the control. Pretreatment of the mice with 30 mg NaF/L in their drinking water, however, increased the MI% more than in the MMC-treated group without NaF exposure.

Table. Effects of mitomycin-C (MMC) (mg/kg bw) alone or in combination with NaF (mg/L) on mitotic indices (MI) and chromosome aberrations in mouse bone marrow cells *in vivo*. Values are mean±SE (SE=standard error)

Group (n = 4)	TM[a]	MI (%)[b]	Ab M (%)[c]	Ctd Br (%)[d]	Iso Ctd Br (%)[e]	Exch (%)[f]
Control	612	2.12±0.06	1.5±0.29	1.5±0.29	0.00	0.00
MMC- 4	595	1.04±0.14*	38±1.08*	48.75±2.06*	0.25±0.25	1.25±0.48*
NaF-7.5	482	2.35±0.26	7.5±0.5*	7.75±0.25*	0.00	0.00
NaF-7.5+MMC-4	411	0.79±0.13*	35±3.03*	49.25±6.39*	0.25±0.25	1±0.71
NaF15	570	1.78±0.39	9.25±0.63*	9.5±0.65*	0.00	1.25±1.25
NaF-15+MMC-4	594	0.99±0.22*	36.25±1.11*	44.75±1.49*	0.5±0.29	0.75±0.48
NaF-30	611	1.85±0.28	8.25±0.85*	8.5±1.19*	0.00	0.00
NaF-30+MMC-4	546	1.48±0.07*	32.25±2.50*	38±4.308*	0.5±0.29	1.5±0.96

*$p < 0.05$, significantly different from control group.

[a]Total metaphases; [b]Mitotic index; [c]Aberrant metaphases; [d]Chromatid breaks; [e]Isochromatid breaks; [f]Exchanges (end-joining of two nonsister chromatids)

The percentage of aberrant metaphases was significantly higher in all the treatment groups, compared to the control. Exposure of the mice to NaF for 30 days was found to protect the bone marrow cells from MMC induced damage, though the effect was not very great. The percentage of chromatid breaks also showed a similar trend, as there was little or no synergistic effect of NaF and

MMC treatment. A 10% decrease in percentage of chromatid breaks occurred in the bone marrow cells of the mice pretreated with 30 mg/L NaF.

An overall tendency of a suppressive effect of NaF pretreatment was noteworthy against MMC-induced damage to the cells, although the differences were not statistically significant.

DISCUSSION

Although Obe and Slacik-Erben reported a suppressive effect of NaF against alkylating agents *in vitro*,[1,2] and Joseph and Gadhia found an increased frequency of MMC-induced chromosome aberrations in one of the three F-endemic populations in Gujarat, India,[3] there appears to be no comparable *in vivo* study on such effects of NaF in an animal model. Our results indicate a reduction in the clastogenic effect of MMC in the bone marrow cells of mice pretreated with NaF. These findings thus support the observations of Obe and Slacik-Erben that the lower rate of alkylation in cellular DNA in the presence of NaF is a possible reason for this suppressive effect. A recent study reported that during combined exposure to sodium arsenite ($NaAsO_2$) and NaF, the toxic effects were less pronounced compared to their individual effects.[5] In some cases, however, clear antagonistic effects were noted following co-exposure to $NaAsO_2$ and NaF. It was therefore suggested that interaction in the gastrointestinal sites may have resulted in an antagonism of arsenite and fluoride. However, our results clearly disagree with other reports not recording any damage to DNA by NaF in cultured cells.[6-8] This discrepancy may be due to differences in sensitivity of different cell types to NaF and/or possible differences in the mechanism of action of NaF *in vivo* and *in vitro* requiring further study.

Gadhia and Joseph observed an increase in human chromosome aberrations but not in sister chromatid exchanges in cultured human lymphocytes exposed to 30 ppm of NaF *in vitro*.[9] In the present study, the mice were treated orally through drinking water to NaF (7.5–30 mg/L), equivalent to the range of F concentrations found in India and other countries. It is clear, therefore, that NaF can significantly increase chromosome aberrations and aberrant metaphase percentages in bone marrow cells of mice *in vivo*. Thus our report is particularly important since NaF, although less used now, was the first chemical employed for fluoridation of drinking water, thereby exposing human populations to the risk of chromosomal lesions.

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