DNA DAMAGE IN THYROID GLAND CELLS OF RATS EXPOSED TO LONG-TERM INTAKE OF HIGH FLUORIDE AND LOW IODINE

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SUMMARY: Thirty-two one-month-old Wistar albino rats were divided randomly into four equal groups of eight (female:male = 3:1). To assess damage to DNA in their thyroid gland cells, the first group (1) of rats served as the untreated control, the second group (2) was administered a high concentration of fluoride (HiF, 100 mg NaF/L [45 mg F^-/L] in their drinking water), the third group (3) was placed on a low iodine intake (LI, 0.0855 mg I/kg diet), and the fourth group (4) was exposed to the high fluoride and low iodine treatment combined (HiF+LI). At 20 months of age, the rats were sacrificed for experimental purpose and their thyroid gland cells were removed for single cell gel electrophoresis (SCGE = comet assay). In comparison with DNA damage in the thyroid gland cells of the control group 1 (10.74 ± 12.59%), such DNA damage in the LI, HiF, and HiF+LI groups 2, 3, and 4, was 83.50 ± 10.20%, 83.03 ± 12.11%, and 89.32 ± 8.21%, respectively. Moreover, the proportion of grade III thyroid gland cell damage increased by 32.26% in group 2, 47.83% in group 3, and 69.23% in group 4, as compared to the control group 1. These findings indicate that excessive long-term intake of fluoride, with or without adequate I intake, is a significant risk factor for the development of thyroid dysfunction.

Keywords: Comet assay; DNA damage; High fluoride intake; Iodine deficiency; Rat thyroid; Single cell gel electrophoresis (SCGE); Thyroid gland cells.

INTRODUCTION

Ordinarily, fluorine, as fluoride ion (F^-), is present in soil and water in low concentrations. However, F^- may represent a threat to public and occupational health when its presence in the environment increases due to natural or anthropogenic sources. Excessive intake of F^- via drinking water is an endemic problem in a number of countries, including, among others, China, India, and Mexico.[1,2]

Some reports showed that fluorosis and iodine deficiency co-exist in some areas.[3-5] The incidence of goiter (30.07%) in children aged 8-14 years in areas of high F^- (>2 mg F^-/L in water) and iodine deficiency was higher than in an iodine-deficient area (10.6%) with 0.8 mg F^-/L. In comparison with the iodine-sufficient control area, loss of IQ was amplified in the iodine-deficient region with 0.8 mg F^-/L in the water supply (IQ loss 8.66%) and even more pronounced in areas of high F^- (>2mg F^-/L) and iodine deficiency (IQ loss 14.43%). The incidence of dental fluorosis also increased concurrently.

The thyroid gland appears to be the most sensitive tissue in the body to F^-,[6] which is able to increase the concentration of thyroid stimulating hormone (TSH) and decrease the concentration of T_3 and T_4 hormones, thereby producing hypothyroidism in some populations.[7] Consequently, prolonged

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consumption of high F^- water has the potential to suppress the function of the thyroid gland.

Single cell gel electrophoresis (SCGE) or comet assay is a simple, rapid, and sensitive technique for measuring DNA damage. The alkaline method for SCGE is a very sensitive assay for the detection of single-stranded breaks in DNA.[8-10]

Although effects of F^- on the thyroid gland have been studied, there has been little examination of F^- damage to DNA in thyroid gland cells. In view of the results of our research on the effects of high F^- and low iodine concentrations on biochemical indexes, histopathology, and DNA damage in the brain and the resultant negative impact on the learning-memory in offspring rats, [11-14] we decided to apply the technique of SCGE to detect DNA damage in thyroid gland cells of rats following long-term exposure to high F^- and low iodine concentrations in their drinking water.

MATERIALS AND METHODS

Experimental materials: As in our recent reports,[11-14] one-month old Wistar albino rats, each weighing approximately 50 g, were obtained from the Experimental Animal Center of Shanxi Medical University for use in this study.

The same iodine-deficient feed and high-fluoride water reported in those previous studies[11-14] were also employed here as shown in Table 1.

Table 1. Fluoride in the drinking water (mg F^-/L) and fluoride and iodine levels in the diet (mg I/kg) of the rats

	Control	High fluoride (HiF)	Low iodine (LI)	High fluoride and low iodine (HiF+LI)
Iodine in diet	0.3543	0.3543	0.0855	0.0855
Fluoride in diet	25.57	25.57	26.01	26.01
Fluoride in drinking water	<0.6	45[a]	<0.6	45[a]

[a]From 100 mg NaF/L as recorded in our four previous reports.[11-14]

Animal test model: The rats were the same as those in our published study.[14] At age 20 months, the rats were sacrificed and their thyroid gland cells were prepared for single cell gel electrophoresis (SCGE).

Preparation of single cell suspension: After the rats were anesthetized with 20% urethane (ethyl carbamate, $NH_2COOC_2H_5$) solution, they were sacrificed by transcardial perfusion with 0.9% saline. Their thyroid glands were then removed, washed three times with phosphate buffer solution (PBS: NaCL 8.0 g, KCl 0.2 g, $Na_2HPO_4.12H_2O$ 2.8 g, and KH_2PO_4 0.2 g; pH 7.4), cut into pieces with stainless steel scissors, homogenized with the appropriate amount of PBS (at 4°C and pH 7.4) in a glass homogenizer at 0–4°C, and then sifted through a 147-μm sieve. The slides were prepared by staining with trypan blue: 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt. Using a microscope, we found that up to 90% of the brain cells were alive. The cells were resuspended at approximately 10^6 cells per mL in PBS and then immediately used for comet assay.

Alkaline comet assay (single cell gel electrophoresis): The same alkaline comet assay reported in our earlier study[14] was also used here.

RESULTS

In comparison with the controls, the ratios of tailing and tail length of thyroid gland cells in the HiF group, the LI group, and the HiF+LI group were significantly increased (Tables 2 and 3), and experimental cells showed the typical comet configurations seen in Figures 1–4. The proportion of grade III (most severe) migrated thyroid gland cell damage was greatest in the HiF+LI group, as shown in the frequency histogram in Figure 5.

Table 2. Ratio of tailing of thyroid gland cells Induced by high fluoride and low iodine (Mean ± SD)

	No. of samples	No. of cells analyzed	Ratio of tailing (%)
Control	5	500	10.74±12.59
Low iodine (LI)	5	500	83.50±10.20*
High fluoride (HiF)	5	500	83.03±12.11*
High fluoride and low iodine (HiF+LI)	6	600	89.32± 8.21*

*p<0.01.

Table 3. Length of tail of thyroid gland cells induced by high fluoride and low iodine (Mean ± SD)

	No. of cells analyzed	Length of tail (μm)
Control	125	3.50±3.23
Low iodine (LI)	125	20.73±13.44*
High fluoride (HiF)	125	29.24±26.88*
High fluoride and low iodine (HiF+LI)	150	42.24±25.93†

*p<0.05; †p<0.01.

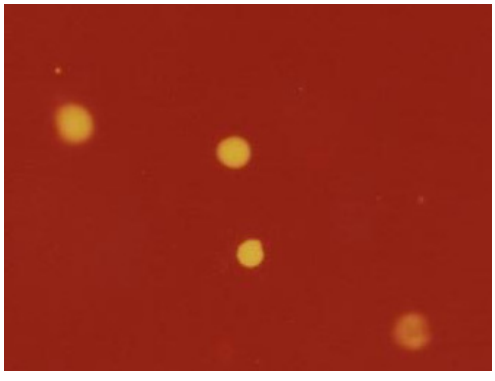


Figure 1. Comet of thyroid gland cells in control.

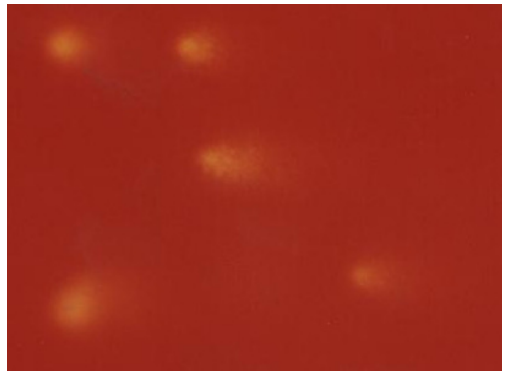


Figure 2. Comet of thyroid gland cells in LI group.

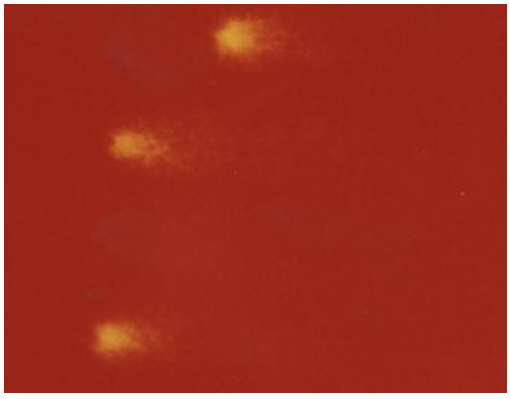


Figure 3. Comet of thyroid gland cells in HiF group.

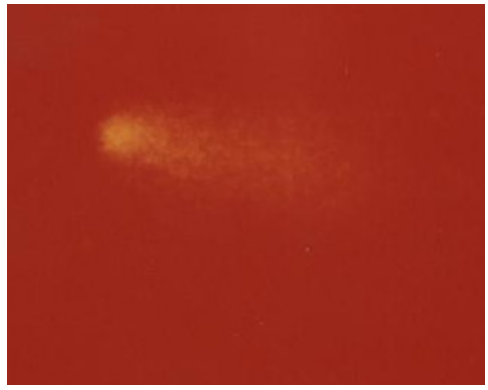
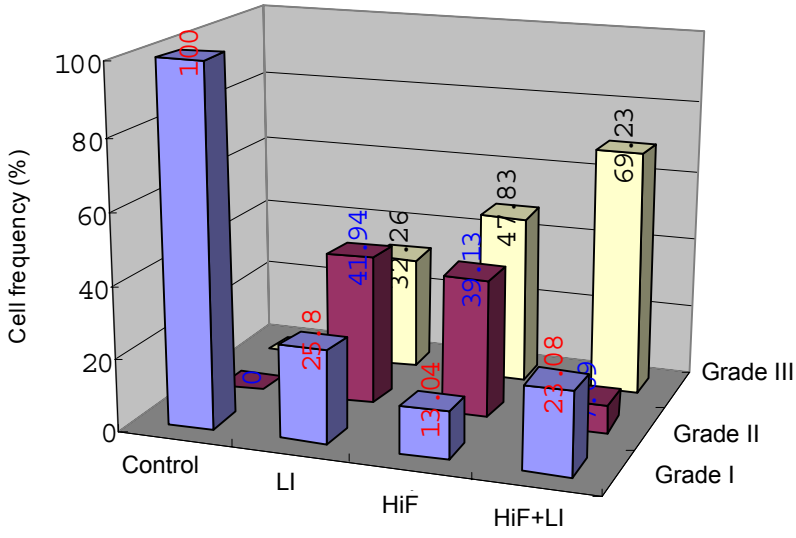


Figure 4. Comet of thyroid gland cells in HiF+LI group.



Column heights (%)	0	32.26	47.83	69.23
	0	41.94	39.13	7.69
	100	25.80	13.04	23.08

Figure 5. Frequency histogram of undamaged and migrated thyroid gland cells according to severity by grade in control and LI, HiF, and HiF+LI groups.

DISCUSSION

As a halogen, fluorine is related chemically to iodine. However, it is very much more reactive chemically than iodine and therefore, even as F^-, can be expected to interfere with iodine in the thyroid gland.[15] Moreover, both iodine and fluorine have antagonistic effects on the thyroid gland. In the 1930s, Goldemberg[16] was the first to introduce fluorine therapy for hyperthyroidism and Basedow's disease on the assumption that simple goiter and cretinism were caused not by iodine

deficiency but by a superabundance of F^- in the air, food and water. Nevertheless, if the iodine content of the water is already very low, comparatively low concentrations of F^- in the drinking water may conceivably aggravate the effects of iodine deficiency on the thyroid.

In the present study, DNA damage of thyroid gland cells exposed to high F^-, low iodine, and the interaction of both factors, markedly increased, especially in the HiF+LI group, with grade III damage up to 69.23%. We suggest that F^- may directly damage cells and induce rupture of DNA strands and thereby cause dysfunction of the thyroid gland. Perhaps DNA damage is one of reasons for the high morbidity rates among those afflicted with hypothyroidism goiter and sub-cretinism in high F^- and low iodine areas. It is noteworthy that iodine deficiency can induce DNA damage of thyroid gland cells in our study. These results also support the findings by Fang et al.[17] showing a high incidence of thyroid cancer (15.6%) in iodine deficient rats and mice compared to a zero percent incidence in controls.

Some reports show that F^- can induce structural changes and dysfunctions in the thyroid gland.[18-22] The thyroid gland has a strong capacity for absorbing and accumulating F^-. The fluorine content in the thyroid gland is reported to be second only to that of the aorta in non-bone tissues.[23] F^- can directly injure the structure of the thyroid follicle and induce cytoplasm reduction and karyopycnosis of follicular epithelial cells, reduce the number of microvilli on the cristae of epithelial cells, and lead to swelling vacuoles in follicular epithelial cells of the thyroid gland.[18,24] Also, F^- disturbs the synthesis and secretion of thyroid hormone, interferes with the activity of enzymes that catalyze the conversion of thyroxine (T_4) into the active thyroid hormone triiodothyronine (T_3) and inactive metabolites, thereby leading to perturbations of circulating thyroid hormone levels.[20,25-28] Furthermore, ingesting excess F^- stresses the functional status of the hypothalamus-pituitary-thyroid system, thus adversely affecting the synthesis of DNA and RNA in thyroid cells.

In conclusion, DNA strands in thyroid gland cell have been shown to be adversely affected when rats are exposed to high F^-, low iodine, and their interactive combination from the age of one month to 20 months. The rate and degree of DNA damage in thyroid gland cells is higher in the HiF+LI group than in either the HiF or LI group. These findings demonstrate that excessive intake and accumulation of F^- in the body is a serious risk factor for the development of thyroid dysfunction, especially when iodine deficiency also exists.

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