TOXIC EFFECTS OF FLUORIDE BY MATERNAL INGESTION ON KIDNEY FUNCTION OF ADULT MICE AND THEIR SUCKLING PUPS

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SUMMARY: To assess renal damage in experimental fluorosis, female Wistar mice were given 500 ppm NaF (226 ppm F^-) in drinking water from the 15th day of pregnancy until the 14th day after delivery. All mice were sacrificed on day 14 after parturition. No significant changes were found in relative kidney weights of fluoride-treated mice and their pups. Urinary fluoride excretion was three times higher in mothers treated with NaF, whereas the rate of fluoride excretion increased by only 3% in their pups. Fluoride administration strongly affected urinary and plasma parameters in 14-day-old mice and their mothers. Daily urine volume in treated groups was higher in the adult mice and their pups than in the controls. Creatinine, a specific indicator of glomerular function, showed significantly higher plasma and lower urinary levels in the treated groups than in the controls. Lipid peroxidation increased in the treated mice, as revealed by high kidney malondialdehyde levels, while plasma and urinary uric acid levels showed a significant decline. There was also a significant increase in urinary zinc and copper levels in both mothers and pups, whereas the plasma levels decreased.

Keywords: Copper; Creatinine; Creatinine clearance; Fluoride-exposed mice; Lipid peroxidation; Malondialdehyde; Renal failure; Uric acid; Zinc.

INTRODUCTION

High exposure to fluoride may occur from natural or industrial sources and from misuse of fluoride-containing dental care products.[1] The most obvious early toxic effects of fluoride on humans are dental and skeletal fluorosis that are endemic in areas with elevated exposure to fluoride. Detrimental effects of high-fluoride intake also affect soft tissues,[2,3] including the liver,[4] lungs,[5] brain,[6] and kidneys.[1] Quite sensitive in their histopathological and functional responses to toxic amounts of fluoride,[7] the kidneys are the primary organ concerned with excretion and retention of fluoride after chronic fluoride intoxication.[8] Numerous structural and functional changes have been noted in kidneys of animals receiving increased amounts of fluoride under different conditions.[9] Fluoride nephrotoxicity causes pathological changes in the glomeruli and in the proximal, distal, and collecting tubules.[10,11] Its effects on glomerular function are less severe, whereas proximal tubular injury is more evident by analysis of urinary biochemical indices such as *N*-acetyl-β-D-glucosaminidase; α-glutathione-S-transferase, and creatinine.[12] Although urinary fluoride excretion has been widely used as an indicator of fluoride intake and exposure,[8] reports concerning urinary biomarkers for the evaluation of kidney damage caused by fluoride are

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very limited. Fluoride-induced polyuria resulting from kidney damage is the main symptom,[13,14] but kidney injury by fluoride might also be explained, at least in part, by lipid peroxidation.

Reports describing kidney intoxication by fluoride in adult mice are scarce and apparently absent in newborn mice. In an effort to better our understanding of the mechanism of fluoride renal toxicity, the present study was designed to investigate the effects of sodium fluoride on kidney function in adult maternal mice and their pups during the suckling period.

MATERIALS AND METHODS

The same protocol was followed as in our previous papers.[15,16] Wistar mice were purchased from the Central Pharmacy of Tunis. They were kept under conditions of controlled temperature (23±2 °C) and a daily 12-hr photoperiod with lighting between 8 am and 8 pm. They had free access to water and a commercial rodent diet (SICO, Sfax, Tunisia). The standard diet contained 0.992±0.014 µg of iodine by gram of diet. Mating dates were established from the appearance of vaginal plugs. The day on which a plug appeared was designated day 0 of pregnancy. Twelve pregnant mice were divided into two groups of 6. The first group represented control animals. The second group was administered 500 ppm of NaF (226 ppm F^-) in their drinking water from day 15 of pregnancy until day 14 after delivery. Pregnant female mice were allowed to deliver spontaneously three weeks after coitus. At birth, litters were reduced to eight pups each within 24 hr after parturition. The day of birth was designated as day 0 of lactation. Urinary samples were obtained from animals housed in a specially designed metabolic cage where fecal contamination was avoided. Urine samples were collected into bottles in 24-hr cycles. Urinary volume of 14-day-old mice was calculated by taking away the 24-hour urine volume average of mothers kept with their pups and those kept alone in metabolic cages. The volume of each sample was recorded and centrifuged at 3000 g for 5 min.

All pups (n = 96) and their mothers (n = 12) were sacrificed on postnatal day 14. After fatal anaesthesia by intra-abdominal injection with chloral hydrate, relative kidney weights were measured and blood samples were collected from the brachial artery and centrifuged at 2200 g. Plasma and urine samples were kept at –20 °C until analysis. Fluoride, zinc, and copper levels were determined in plasma and urine using, respectively, an ion specific electrode[17] and atomic absorption. Urinary and plasma levels of creatinine and uric acid were measured using, respectively, the colorimetric method (Biomaghreb kits ref: 20151) and uricase colorimetric test (Biomaghreb kits ref: 20091). Creatinine clearance was calculated by the equation:[18]

$$\text{Creatine clearance} = \frac{U \times V}{P}$$

where U is urinary creatinine level, V is the volume of urine sample collected within 24 hours and P is the plasma creatinine concentration. Lipid peroxidation

in kidneys was assessed by measuring malondialdehyde (MDA) levels in kidney homogenates.[19]

Comparisons of mean values between treated and control animals were made using Student's t test.[20] Statistical significance was defined as a p value of less than 0.05.

RESULTS

Compared with the controls, the NaF-treated mothers and their pups had a significant decrease in body weight (–19.1 and –15.9%, respectively; p≤0.001) (Table 1). On the other hand, no significant changes were found in the relative kidney weights of adult mice treated with fluoride and their pups compared with those of the controls (Table 1).

Table 1. Effect on body weight and relative kidney weight of 14-day-old mice and their mothers administered NaF in the drinking water (500 ppm) of the mother from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth

Group	Body weight (g)		Relative kidney weight (mg/g bw)	
	Controls (n=10)	NaF groups (n=10)	Controls (n=25)	NaF groups (n=25)
Mothers	41.445±1.681	33.528±1.863*	5.671±0.158	5.815±0.123
Offspring	8.996±0.445	7.563±0.387*	6.732±0.518	6.581±0.764

Significant differences: NaF groups vs controls: *p≤0.001.

In adult mice exposed to NaF for 20 days, urinary fluoride levels were three times higher than in control group (p≤0.001). In their pups the rate of fluoride excretion increased by 3% (Figure 1).

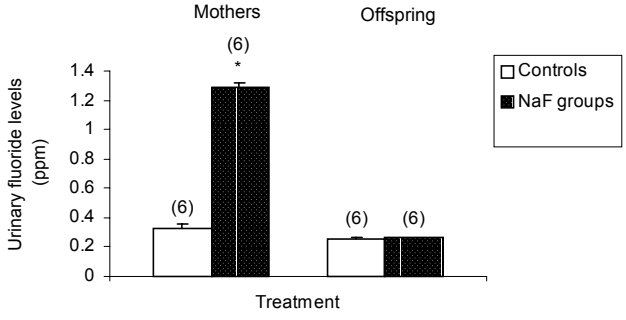

Figure 1. Effect on urinary fluoride levels of adult mice and their pups administered NaF in the drinking water (500 ppm) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth. Significant differences: NaF groups vs controls: *p≤0.001

The daily urine volume in treated mothers and in their pups was higher than in the controls (+36 and +69%, respectively; p≤0.001) (Figure 2). Compared to the controls, creatinine levels in the treated dams were 82% higher in the plasma and 80% lower in the urine; in their pups they were 59% higher in the plasma and 86% lower in the urine (Tables 2 and 3). Creatinine clearance was 82% lower in the treated adult mice and 85% lower in their pups (p≤0.001) (Figure 3).

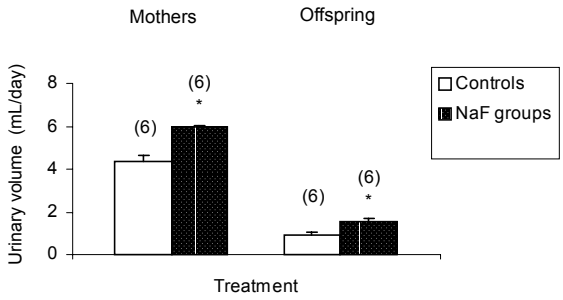

Figure 2. Effect on urinary volume of adult mice and their pups administered NaF in the drinking water (500 ppm) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth.Significant differences: NaF groups vs controls: *p≤0.001.

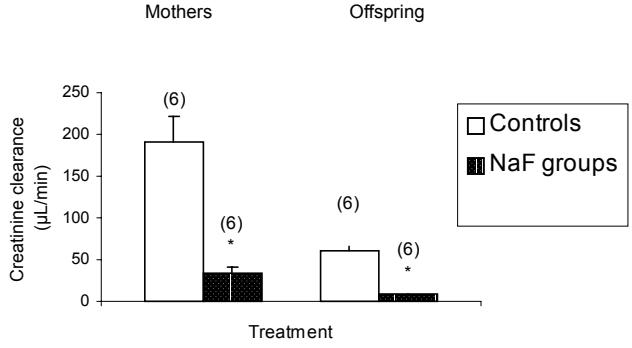

Figure 3. Effect on creatinine clearance of adult mice and their pups administered NaF in the drinking water (500 ppm) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth. Significant differences: NaF groups vs controls: *p≤0.001.

In kidney homogenates of the test mice, MDA levels were significantly increased compared to those of the controls (+41% in mothers and +29% in pups) (Figure 4).

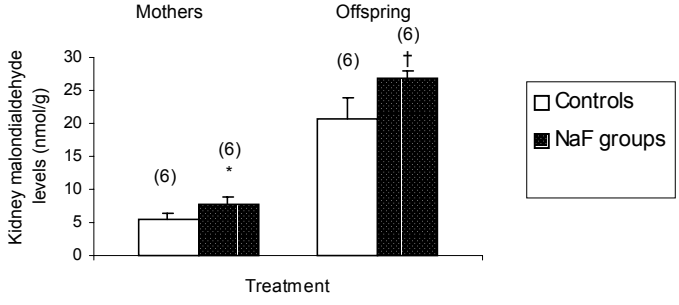

Figure 4. Effect on kidney malondialdehyde levels of adult mice and their pups administered NaF in the drinking water (500 ppm) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth.
Significant differences: NaF groups vs controls: *p≤0.01; †p≤0.001.

Table 2. Effect on plasma levels of creatinine, uric acid, zinc, and copper in 14-day-old mice and their mothers administered NaF in the drinking water (500 ppm) of the mother from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth

Group	Plasma creatinine level (µmol/L)			Plasma uric acid level (µmol/L)		
	Controls (n=6)	NaF groups (n=6)	Normal adults	Controls (n=6)	NaF groups (n=6)	Normal adults
Mothers	90.66±17.04	164.83±8.79†		266.56±3.51	104.11±7.83†	
Offspring	89.67±2.94	142.25±14.42†		250.66±27.79	78.33±12.46†	
Normal adults			102 ±22[a]			218.89 ±4.76[b]

Group	Plasma zinc level (µg/dL)			Plasma copper level (µg/dL)		
	Controls (n=6)	NaF groups (n=6)		Controls (n=6)	NaF groups (n=6)	
Mothers	126.66±9.83	89.16±17.44*		162.51±20.91	116.67±12.91*	
Offspring	85.01±3.53	12.04±1.87†		180.03±18.71	137.51±20.91*	
Normal adults			110 ±11[c]			100 ±50[d]

Significant differences: NaF groups vs controls: *p≤0.01; †p≤0.001.

a, b, c and d: normal range in adult mice of plasma creatinine, uric acid, zinc and copper levels respectively according to following references:

a: Mitra A, Chakraborty S, Auddy B, Tripathi P, Sen S, Saha AV, et al. Evaluation of chemical constituents and free-radical scavenging activity of Swarnablasma (gold ash), an Ayurvedic drug. J Ethnopharmacol 2002;80:147-53.

b: Kong LD, Yang C, Ge F, Wang HD, Guo YS. A Chinese herbal medicine ermiao wan reduces serum uric level and inhibits liver xanthine dehydrogenase and xanthine oxidase in mice. J Ethnopharmacol 2004;93:325-30.

c: Mocchegiani E, Giacconi R, Cipriano C, Gasparini N, Orlando F, Stecconi R, et al. Metallothioneins (I+II) and thyroid-thymus axis efficiency in old mice: role of corticosterone and zinc supply. Mech Ageing Dev 2002;123:675-94.

d: Davis GK, Mertz W. Copper. In: Mertz W, editor. Trace elements in human and animal nutrition. New York: Academic Press;1987. p.301-50.

Uric acid levels were 61% lower in the plasma of the treated dams and 63% lower in their urine; in their pups the decreases were 69% and 57%, respectively (Tables 2 and 3). Significant increases in urinary zinc and copper levels were observed in the treated dams (+80 and +43%, respectively) and in their pups (+76 and +65%, respectively) (Table 3). On the other hand, plasma levels of zinc and copper were lower in the dams (–30 and –28%, respectively) and in their pups (–86 and –24%, respectively) (Table 2).

Table 3. Effect on urinary levels of creatinine, uric acid, zinc, and copper in 14-day-old mice and their mothers administered NaF in the drinking water (500 ppm) of the mother from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth

Group	Urinary creatinine level (µmol/L)		Urinary uric acid level (µmol/L)	
	Controls (n=6)	NaF groups (n=6)	Controls (n=6)	NaF groups (n=6)
Mothers	6406±821	1261±321†	1223±93	454±49†
Offspring	8300±283	1133±147†	1843±74	798±63†
Group	**Urinary zinc level (µg/dL)**		**Urinary copper level (µg/dL)**	
	Controls (n=6)	NaF groups (n=6)	Controls (n=6)	NaF groups (n=6)
Mothers	17.08±1.88	30.83±4.92†	116.66±12.91	166.67±25.82*
Offspring	13.12±2.59	23.12±3.47†	83.33±20.41	137.51±13.69†

Significant differences: NaF groups vs controls: *$p \leq 0.01$; †$p \leq 0.001$.

DISCUSSION

Since the kidney is a main target organ of mammalian fluoride intake,[21] renal toxicity can occur after acute and chronic fluoride intoxication. [22,23]

After fluoride treatment, rats exhibit numerous functional impairments[7] and morphological modifications[24] of their kidneys. Al-Hiyasat *et al*[24] observed a significant increase in both absolute and relative kidney weights compared to controls in adult female rats after NaF ingestion. In the present study, however, no significant changes were found in relative kidney weights of the test groups, in agreement with results of Chen *et al*,[5] who exposed mice to airborne fluoride. Along with biochemical changes in mice with 500 ppm of NaF in their drinking water, we noted a significant increase in urinary fluoride excretion in mothers which occurs almost exclusively via the kidney,[25,26,27] since fluoride is rapidly filtered into the urine by the kidney.[9,28] On the other hand, urinary fluoride levels in the nursing pups did not differ between the test and control groups, in accord with the finding that limited transfer of fluoride from plasma to breast milk occurred when 1–5 mg of fluoride was given orally to lactating women.[29] After higher intake of fluoride (25 mg of NaF/day), lactating mothers had a slight increase in fluoride concentration in their breast milk.[30]

Fluoride administration to mice in the present study strongly affected plasma and urinary parameters such as the 24-hour urine volume, creatinine levels, and creatinine clearance. In fact, our results demonstrated that urinary volume was significantly increased after fluoride treatment. This phenomenon is likely to

have been induced by inhibition of salt and water resorption in the proximal tubules[31] and in the thick ascending limb of Henle's loop,[32,33] by increased blood flow to the renal medulla,[34] and by decreased responsiveness of the collecting duct to vasopressin.[35] Polyuria is also explained by the inhibition of antidiuretic hormone secretion, which normally provokes water reabsorption across the collecting duct.[26] In the present investigation, declines in the 24-hr urinary excretion of creatinine and of creatinine clearance were used, respectively, as qualitative and quantitative indexes of the alteration in glomerular filtration rate (GFR), in agreement with the results obtained by Jolly *et al*,[36] who had found a reduction in creatinine clearance of patients with skeletal fluorosis. The GFR decrease may also have been brought about by a rise in preglomerular or a fall in postglomerular resistance and a rise in renal vascular resistance.[23] These biochemical alterations could be correlated with histological changes in the kidney of mice (unpublished results in our laboratory) which confirmed data reported recently for rabbits exposed to high doses of NaF. [37]

Fluoride is also known to cross cell membranes and to enter into soft tissues such as the liver, brain, and kidneys.[38,39] Impairment of soft tissue function has been demonstrated in fluoride-intoxicated animals[40,41] by enhanced lipid peroxidation and decreased activities of antioxidant enzymes. Our studies showed that mice, after fluoride treatment, exhibit a significant increase in renal MDA levels and a decrease in plasma antioxidant defense system such as uric acid, zinc, and copper plasma levels. These results confirm findings by others of a close association between fluoride toxicity and increased oxidative stress in humans[42,43] and in experimental animals.[41,44,45] Shivarajashankara *et al*[46] reported that chronic fluoride toxicity of children elicited increased lipid peroxidation. This peroxidation is associated with free-radical toxicity mediated by oxidative stress manifested by increased levels of MDA and decreased levels in activities of antioxidants in the blood. In plasma, uric acid is the most important antioxidant,[47] and its plasma level decreased in our treated mice as observed in fluorotic children.[46] Plasma copper and zinc, cofactors of superoxide dismutase, (SOD-Cu-Zn), an antioxidant enzyme,[48] significantly decreased after NaF treatment. These results suggest that fluoride toxicity may involve a reduction in some intrinsic scavengers resulting in an increased vulnerability to oxygen free-radical toxicity.

In conclusion, we found that ingestion of fluoride by mice provoked renal damage in both dams and suckling pups.

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