EFFECTS OF FLUORIDE EXPOSURE ON THE ANTIOXIDATIVE STATUS IN THE KIDNEYS OF OFFSPRING MICE DURING THE EMBRYONIC AND SUCKLING PHASES

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ABSTRACT: Long term fluoride exposure can lead to renal oxidative stress in adult animals and humans. However, the toxic effect of fluoride on the antioxidative status in the kidneys in infant animals and humans exposed to fluoride only through cord blood and breast milk remains largely unknown. In the present study, female mice were treated with 25, 50, and 100 mg/L sodium fluoride (NaF) in their drinking water from pregnancy day 0 to day 21 after delivery, and the levels of the enzymatic antioxidants in the kidneys of their offspring at postnatal day 21 were measured. The results showed that, in the high fluoride group compared with the control group, there was a significant increase (p<0.05) in the malondialdehyde (MDA) content and a significant decrease (p<0.05) in the catalase (CAT) activity but no statistical differences were observed in the activities and mRNA expressions of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) in all the fluoride treatment groups. Meanwhile, the CAT mRNA level was increased (p<0.05) in the low and medium fluoride groups, compared with the control. The findings suggest that CAT could be more sensitive, than SOD, GPx, and GR, to fluoride exposure in early life.

Keywords: Antioxidative status; Catalase; Embryonic and suckling phases; Kidney; Offspring mice.

INTRODUCTION

There are two main types of environmental fluoride pollution: geographical high fluoride exposure and artificial fluoride contamination. The former one results from fluoride gathering at certain geographical locations in the crust,1 while the later one is mainly due to human activities, including (i) industrial pollution from the coal industry,2 electric power plants,3 and steel refiners,4 (ii) water contamination produced by the use of fluoride-containing chemicals in agriculture, such as insecticides and phosphate fertilizer, and the use of wood preservatives,5 and (iii) daily fluoride consumption from drinking water, tea, food, toothpaste, and mouthwash products.6

Fluoride can be readily absorbed by the intestine, with entry into the plasma, and distribution to various tissues and organs.7 Therefore, it can be detected in diverse body fluids, such as plasma, cord blood, breast milk, and urine. The largest tissue stores of fluoride in the body are in bone and teeth. The kidneys are the main organ for fluoride excretion. Urinary excretion accounts for 50–80% of fluoride elimination.8 Previous studies on adults reported that high fluoride levels could disturb kidney function and led to renal histopathological changes.9 Although the

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embryonic stage is crucial to the development of the kidneys, the effects of fluoride exposure during gestation and lactation on kidney are still unclear. In the present study, the antioxidative status in the kidneys was evaluated in the mouse pups exposed to fluoride during the embryonic and suckling periods.

**MATERIALS AND METHODS**

**Establishment of the animal model:** Forty-eight adult Kunming mice (male:female =1:1), weighing approximately 20–25 g, from Experimental Animal Center of Shanxi Medical University were fed a standard diet. The animals were all kept in cages bottomed with shavings as bedding material, and had free access to food and water under the standard conditions of a temperature of 22–25°C, and a 12/12-hr light/dark cycle. After one week, the female and male mice were caged together for mating at the ratio of one to one. When the vaginal plug was observed, the females were kept individually, and considered to be at pregnancy day 0. All the pregnant female mice were randomly divided into four groups. The control group was given distilled water. The remaining three groups were given 25, 50, or 100 mg/L NaF in their drinking water from pregnancy day 0 to day 21 after delivery, and labeled as low fluoride, medium fluoride, and high fluoride groups, respectively. The experiment was approved by the Institutional Animal Care and Use Committee of Shanxi Agricultural University.

**Haematoxylin and eosin (HE) staining:** After cervical dislocation at postnatal day 21, the mouse kidneys were quickly removed. A portion of kidney from each mouse was fixed in formalin solution for 72 hr and rinsed with distilled water for 24 hr. It was then dehydrated in graded alcohol, embedded in paraffin, and stained with HE for histological examination.

**Biochemical assays:** The kidney tissues were homogenized and centrifuged at 5,000 rpm for 10 min to get the supernatants. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities and malondialdehyde (MDA) content were determined following the instructions with the commercial test kits (Jiancheng Biotech, Nanjing, China). The measurements were conducted in triplicate for each sample by using Thermo Scientific Varioskan Flash Multimode Reader (Thermo Fisher Scientific, USA). The total protein content of each sample was examined by the method of Bradford in order to normalize the activities of SOD, CAT, GPx, and GR and the level of MDA.

**RNA extraction and quantitative real time PCR (QRT-PCR):** The kidney total RNA was isolated following the Trizol manufacturer’s instructions. The quality and quantity of the RNA were assessed by gel electrophoresis and a Nanodrop ND-2000 spectrophotometer (Nanodrop Technologies Inc., DE, USA). The primers were designed with Primer 3.0 plus and the sequences are shown in the Table. QRT-PCR was conducted on the Stratagene Mx3000P™ QRT-PCR system (Stratagene, La Jolla, CA, USA) using SYBR Premix Ex Taq™ II kit (Takara, Dalian, China). The PCR cycling conditions were as follows: after initial denaturation at 95°C for 10 sec, 45 cycles started at 95°C for 5 sec, 60°C for 15 sec, and 72°C for 6 sec. The conditions for the dissociation curve were 95°C for 1 min, 55°C for 30 sec, and 95°C for 30 sec.
Statistical analysis: The analyses of the abundance of mRNA for SOD-1, CAT, GPx1, and GR were performed by using the comparative ∆∆CT method provided by the Mx3000P™ QPCR system. The rest of the data were analyzed by one-way ANOVA using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, USA). Data were expressed as mean±SE. p< 0.05 was considered significant.

RESULTS

Changes of renal histology: Representative photographs of the kidney histology are shown in Figure 1.

Table. Primer sequences and their corresponding PCR product size.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Product size (bp)</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>F: AGCCATGTACGCTAGCCATCC R: CTCTCAGCTGGTGTTGAGAA</td>
<td>228</td>
<td>NM_007393</td>
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<tr>
<td>SOD-1</td>
<td>F: AGATGACTTGGGCAGAAAGTG R: AATCCCAATCCTCCACAGG</td>
<td>85</td>
<td>NM_011434</td>
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<tr>
<td>CAT</td>
<td>F: CCTCGTTCAGATGGTGGTTT R: TCTGTTGATATCGTGGTGTA</td>
<td>150</td>
<td>NM_009804</td>
</tr>
<tr>
<td>GPx1</td>
<td>F: TGCGAAGTGAATGGTGAGAA R: AAAGTTCCAGGCAATGTCGT</td>
<td>130</td>
<td>NM_008160</td>
</tr>
<tr>
<td>GR</td>
<td>F: TTATCCACGGCTATGCAACA R: TCTGCTCTCGTGGAGAATC</td>
<td>129</td>
<td>NM_010344</td>
</tr>
</tbody>
</table>

Figure 1. Histopathological photographs of kidney in mice from control (A), low (B), medium (C), and high (D) fluoride groups. Arrows indicate erythrocyte infiltrations. The magnification is × 400.
The control group illustrated a normal histological structure while in the three fluoride treatment groups, although the renal cells presented an intact structure and regular arrangement, variable degrees of erythrocyte infiltration were observed.

Activities of SOD, CAT, GPx, GR, and MDA content in kidney: Compared to the control group, no statistically significant difference was observed in the activities of SOD, GPx, and GR in the kidney of mouse pups with 25, 50, and 100 mg/L NaF maternal exposure during their embryonic and suckling stages (Figures 2–4).

![Figure 2. Activity of superoxide dismutase (SOD) in the kidney of mice exposed to fluoride during their embryonic and suckling phases. Compared to the control: *p<0.05. Values are mean±SE.](image1)

![Figure 3. Activity of glutathione peroxidase (GPx) in the kidney of mice exposed to fluoride during their embryonic and suckling phases. Compared to the control: *p<0.05. Values are mean±SE.](image2)
Compared to the control group, there was a significant decrease in CAT activity in the high fluoride group (Figure 5).

**Figure 4.** Activity of glutathione reductase (GR) in the kidney of mice exposed to fluoride during their embryonic and suckling phases. Compared to the control: *p<0.05. Values are mean±SE.

**Figure 5.** Activity of catalase (CAT) in the kidney of mice exposed to fluoride during their embryonic and suckling phases. Compared to the control: *p<0.05. Values are mean±SE.
Compared to the control group, the MDA content was markedly enhanced in the high fluoride group (Figure 6).

![Figure 6. Malondialdehyde (MDA) content in the kidney of mice exposed to fluoride during their embryonic and suckling phases. Compared to the control: *p<0.05. Values are mean±SE.](image)

**mRNA expression of SOD, CAT, GPx, and GR in kidney:** Compared to the control group, the CAT mRNA level was significantly elevated in the low and medium fluoride groups. The mRNA expressions of SOD, GPx, and GR were not significantly different in the three fluoride treatment groups (Figure 7).

![Figure 7. The mRNA expressions of superoxide dismutase1 (SOD1), catalase (CAT), glutathione peroxidase1 (GPx1), and glutathione reductase (GR) in the kidney of mice exposed to fluoride during their embryonic and suckling phases. Compared to the control: *p<0.05. Values are mean±SE.](image)
DISCUSSION

The kidney is a vital organ for eliminating body waste, regulating the pressure, composition, and volume of the blood, and maintaining bone density. These diverse functions require precise renal tubular structures assembled by functionally compartmentalized epithelia which are developed during the fetal period. Due to its responsibility for fluoride elimination, kidney health is threatened by fluoride toxicity. Numerous studies have reported different histopathologic characteristics in the kidney of animals administered various doses of fluoride. In rats, exposure to 50 mg NaF/L for 40 days induced tubular flattening, loss of the proximal tubule brush border, and cell detachment. In mice, blood filled spaces, disintegration of tubular epithelium, and atrophy of glomeruli were present after treatment with 15 mg NaF/L for 90 days. Additionally, 5 mg NaF/L for 500 days caused hypertrophy and hyperplasia in the renal tubules of rats. However, the renal injuries mentioned above were in adult fluoride-intoxicated animals. In the present experiment, the histological structures in kidney of offspring mice were studied at postnatal day 21 after exposure to fluoride only through the cord blood and breast milk. The results showed that although no primary renal structural alterations occurred in mice whose dams were treated with 25, 50, and 100 mg NaF/L, varying degrees of erythrocyte infiltration were observed in all three fluoride groups, indicating that the adverse effect of fluoride on the kidney occurred early in life.

Oxidative stress is a well accepted mode of fluoride intoxication in many tissues and organs. There naturally exists endogenous reactive oxygen species (ROS), which are generated from normal biochemical events, and antioxidant defense systems, comprising enzymatic and nonenzymatic antioxidants for protecting cells by eliminating ROS. Fluoride exposure can disrupt the balance between them through increasing the generation of ROS, lipid peroxidation, and altering the activities of antioxidative enzymes. Oxidative stress evoked by fluoride can be mitigated by antioxidant therapeutics. On the other hand, the kidney is sensitive to oxidative stress which plays a key role in the initiation and progression of renal injury. Previous investigations have reported increased MDA and decreased SOD, CAT, and GPx in the kidney of adult animals exposed to fluoride. In the present study, the maternal fluoride exposure through cord blood and breast milk induced a significantly higher MDA level (p<0.05) and lower CAT activity (p<0.05) in the offspring mice. However, no effects were detected on the activities of SOD, GPx, and GR. Notably, CAT gene expression was significantly elevated by fluoride exposure, which could be the result of compensation for its low activity level.

CONCLUSION

The administration of fluoride to pregnant and lactating mice resulted in an increased MDA level and decreased CAT activity in the kidneys of their suckling pups, and CAT may be more sensitive to fluoride toxicity than SOD, GPx, and GR in early life.
ACKNOWLEDGMENTS

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