# TOXIC EFFECTS OF FLUORIDE ON KIDNEY FUNCTION AND HISTOLOGICAL STRUCTURE IN YOUNG PIGS

Xiu-An Zhan,[a] Min Wang,[a,b] Zi-Rong Xu,[a] Jian-Xin Li[a]

Hangzhou, China

**SUMMARY: The effects of chronic fluoride exposure on kidney integrity and histological structure, along with effects on associated enzymes and metabolite changes, were investigated in young pigs. Twenty-four crossbred barrows (Duroc×Landrace×Yorkshire) about 50 days old were randomly divided into three groups of eight pigs each. Groups I, II, and III received the same basal diet additionally supplemented, respectively, with 0, 100, and 250 mg $F^-$/kg (from NaF). Results obtained after 50 days indicated that supplemental fluoride-treatment caused severe renal histological changes as well as increased renal cell apoptosis. In kidney tissue, lactate dehydrogenenase (LDH) activity was significantly increased in group III, whereas alkaline phosphatase (AKP) activity was significantly decreased in group II as well as in group III. In the serum, significantly increased urea nitrogen (UN) was present in groups II and III, and the serum of group III had elevated creatinine (Cre) and decreased $Na^+$. These findings show that chronic excessive fluoride exposure is deleterious to kidney structure and function of pigs.**

Keywords: Alkaline phosphatase; Creatinine; Fluoride; Kidney function; Kidney histology; Lactate dehydrogenenase; Serum sodium; Serum urea nitrogen; Young pigs.

## INTRODUCTION

The kidney is well recognized for its histopathological and functional responses to excessive amounts of fluoride.[1] Many studies have shown that elevated concentrations of fluoride can occur in the kidney.[2,3] Moreover, this organ is the major route for removal of fluoride from the body[4] and thus is sensitive to renal damage.[5]

Apoptosis is a pathway of cell death, and excessive apoptosis leads to various kinds of organ injury.[6,7] *In vivo* liver lesions from fluoride through apoptosis have been reported,[8] but few studies are available that show fluoride induces kidney lesions through apoptosis.[9]

The aim of this study was to evaluate toxic effects of fluoride on renal function and histological structure in young pigs through a combination of strict morphological criteria, *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, and appropriate biochemical assays.

## MATERIALS AND METHODS

*Animals and their treatment:* Twenty-four 50-day-old barrows (Duroc×Landrace×Yorkshire) with an average body weight of about 17 kg were allotted randomly to three groups. These pigs were the same ones we used earlier.[8,10-11] Group I was the control group, while groups II and III were administered graded doses of reagent grade sodium fluoride (NaF powder provided by Juhua Group Co. Ltd, Zhejiang, China), added to the basal diet at 100 and 250 mg $F^-$/kg, respectively. This diet was a balanced commercially available feed formulated to meet or exceed requirements suggested by the US National

[a]Feed Science Institute, College of Animal Science, Zhejiang University, No. 268 Kaixuan Road, Hangzhou, China, 310029. [b]For correspondence: Min Wang,
E-mail: wing_mail@hotmail.com

Research Council.[12] The fluoride content of the untreated basal diet was 6.2 mg $F^-$/kg, and the drinking water contained 0.6 mg $F^-$/L. The animals were caged in groups of eight at 15–22ºC and were given *ad libitum* access to feed and water. The total test period lasted 50 days.

*Tissue sampling and processing:* At the end of the feeding trial, all pigs were deprived of feed for 12 hr and then slaughtered under general anaesthesia using halothane. Kidneys were dissected out carefully, blotted free of blood, resected and immersed in 10% phosphate buffer formalin and in 2.5% phosphate buffer glutaraldehyde (pH 7.4) for histology and for transmission electron microscopy observation, respectively. In addition, samples of kidney and serum were immediately processed in liquid nitrogen and then stored at –70°C for the assays described as follows.

*Kidney histology:* After fixation for 24 hr, kidney tissues were dehydrated, embedded in paraffin, sectioned at 7 μm thickness, and stained with haematoxylin and eosin (HE) for histological examination. For assessment of apoptosis by the TUNEL procedure, samples of kidney were embedded in paraffin and processed as recommended by the manufacturer of the kit employed (Trevigen, Gaithersburg, MD). Images of kidney sections processed by HE and TUNEL reaction were captured with a Leica DMR microscope equipped with JVC TK-C1380 color video camera. [8]

*Transmission electron microscopy:* The kidneys were treated according to the standard procedure.[13] Briefly, kidney blocks were trimmed to 1×1×1-mm size and washed, quenched, postfixed with ethanol, dehydrated into propylene oxide, and embedded in epoxy resin for transmission electron microscopy studies. Thin sections were cut on Reichert-Jung Ultracut E, then stained with uranyl acetate and lead citrate, and finally examined with a JEM-1230 (JEOL, Tokyo, Japan). [8,12]

*Biochemical assays:* Alkaline phosphatase (AKP) and lactate dehydrogenenase (LDH) activity, and serum creatinine (Cre), urea nitrogen (UN), $Na^+$, and $Cl^-$ were determined according to the procedures recommended by the manufacturer of the kits employed (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

*Statistics:* The experimental data were analyzed with SAS6.12 software and performed using one-way analysis of variance (ANOVA) on files procedure with significance set at $p<0.05$. The analytical data are presented in the following tables as means ± S.D.

## RESULTS

*Effects of fluoride on renal function:* As seen in Table 1, LDH activities in group III were significantly increased, while AKP activities in groups II and III were significantly decreased (all with $p<0.01$). Table 2 shows the significantly increased serum UN in group II ($p<0.05$) and group III ($p<0.01$), elevated serum Cre in group III ($p<0.05$), and decreased serum $Na^+$ in group III ($p<0.05$). A small decreasing trend of serum $Cl^-$ in groups II and III was also observed.

**Table 1.** Effects of fluoride on the activity of renal alkaline phosphatase (AKP) and lactate dehydrogenenase (LDH) in young pigs after 50 days. Values are mean±S.D. (n=8).

| Groups | I | II | III |
|---|---|---|---|
| LDH (Units/mg protein) | 3680.82±618.54 | 3274.85±517.61 | 4464.26±563.80* |
| AKP (Units/mg protein) | 217.41±60.96 | 161.81±30.65* | 139.03±32.21* |

*p< 0.01 compared with group I control.

**Table 2.** Effects of fluoride on serum urea nitrogen (UN), creatinine (Cre), $Na^+$, and $Cl^-$ in young pigs after 50 days. Values are mean±S.D. (n=8).

| Groups | I | II | III |
|---|---|---|---|
| UN (mmol/L) | 3.96±0.68 | 5.00±1.18* | 5.47±0.90† |
| Cre (mmol/L) | 83.02±37.27 | 85.37±25.10 | 116.55±30.94* |
| $Na^+$ (mmol/L) | 168.55±9.62 | 163.55±5.82 | 157.97±12.42* |
| $Cl^-$ (mmol/L) | 109.44±12.05 | 107.31±12.03 | 108.27±2.41 |

*p<0.05, †p< 0.01 compared with group I control.

*Effects of fluoride on kidney histology:* Figure 1 shows how fluoride caused various renal histological structure changes in groups II and III, such as necrosis of glomeruli and tubules, atrophic glomeruli, glomerular capsule and tubules dilatation; moreover, severe tubular leakage appeared in group III.

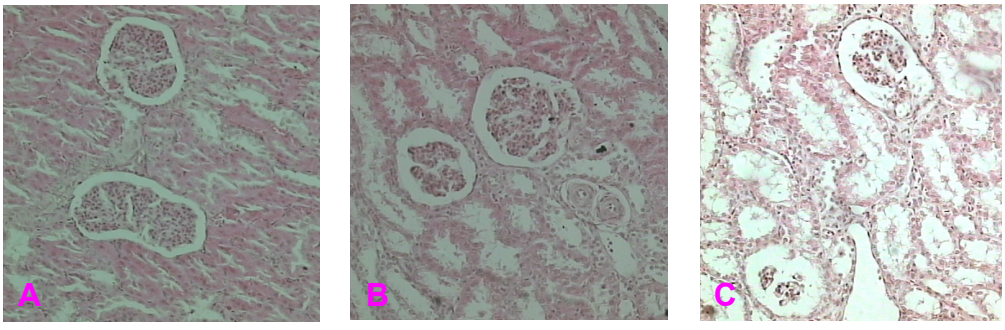


**Figure 1.** Morphological changes of kidney tissue stained with haematoxylin and eosin (HE). A, B, and C: sections of kidney tissues from group I, II, and III, respectively (×400).

Figure 2 indicates that fluoride induced various degrees of damage to the architecture of proximal tubular epithelia, such as cell swelling and lysis, cytoplasm and mitochondria vacuolation, nuclear membrane breakdown, cell shrinkage, nuclear condensation, apoptosis, and necrosis.

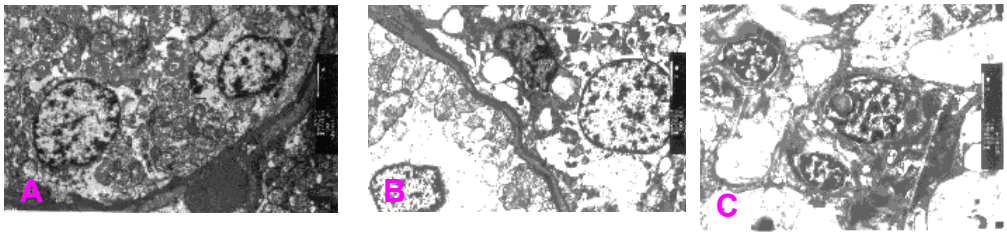


**Figure 2.** Morphological studies by transmission electron microscopy on proximal tubular epithelium integrity. A, B, and C: sections of kidney tissues from group I, II, and III, respectively (×4,000).

Figure 3 suggests that fluoride caused severe impairment of proximal tubule brush border, such as shrinkage, loss, swelling, and vacuolation.


**Figure 3.** Morphological studies by transmission electron microscopy on proximal tubule brush border. A, B, and C: sections of kidney tissues from group I, II, and III, respectively (×15,000).

Figure 4 reveals the relationship between the number of cells enduring apoptosis and the doses of fluoride administered. The number of cells experiencing apoptosis was constantly increasing in the experimental groups II and III.

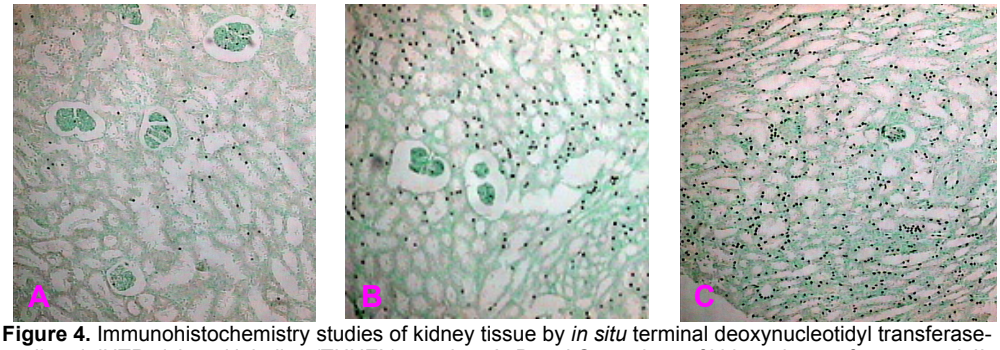


**Figure 4.** Immunohistochemistry studies of kidney tissue by *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) reaction. A, B and C: sections of kidney tissues from group I, II, and III, respectively (×100).

## DISCUSSION

Changes in serum UN, Cre, $Na^+$, and $Cl^-$ are associated with impairment of renal function.[14] The elevated serum levels of UN and Cre and the decreased serum levels of serum $Na^+$ and $Cl^-$ indicate reduced ability of the kidney to eliminate the toxic metabolic substances and reabsorb the metal and non-metal ions. Changes in serum biochemical parameters are consistent with renal histological structure changes. From the observation of the fluoride-treated groups under light microscopy, we find atrophic glomeruli, glomerular capsule and tubule dilatation, and severe tubule leakage. These findings are in agreement with previous fluorosis reports concerning rabbits, rats, and cows.[5,15-18]

To evaluate the cellular morphological architecture and metabolism impaired by fluoride, we examined the proximal tubular epithelium morphological changes as well as the activity of renal LDH and AKP. From the observation of samples of the fluoride-treated groups under transmission electron microscopy, we find damage to the apical brush border membrane of the proximal tubular epithelium including the mitochondria, to the cell and nuclear membrane, and to the cellular organelles. All these degenerative changes are consistent with the observed pattern of activity changes of renal LDH and AKP. Increased activity of LDH is a characteristic feature of a shift from aerobic to anaerobic metabolism leading an elevated rate of pyruvate conversion into lactate, resulting in lactic acidosis.[19-20] AKP exists practically in all tissues, especially on or in the cell membrane.[21] Decreased

activity of renal AKP suggests increased cell membrane permeability and release of AKP within the cell membrane.

It is well accepted that a large number of cells enduring apoptosis will lead to organ lesion. TUNEL assay, an effective method for apoptosis observation, is widely used to determine apoptotic cells.[8,22-23] Figure 4 indicates that large numbers of kidney cells in the group II and III pigs are undergoing or have finished a program of cell death, thus resulting in kidney lesion.

In summary, these studies have shown that fluoride causes various histological structure changes of the kidney, including extensive induction of cell apoptosis, resulting in impairment of renal function and metabolism. These results therefore provide valuable insight on the effects of chronic fluorosis in pigs.

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