

TOXIC EFFECTS OF FLUORIDE ON RABBIT KIDNEY

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Summary: The purpose of this study was to assess renal damage in experimental fluorosis. Young albino rabbits were injected with 5, 10, 20, and 50 mg NaF/kg body weight/day for fifteen weeks and then sacrificed. No significant clinical signs of toxicity were found in animals exposed to the lowest dose. At the higher doses, however, the cytoarchitecture of the kidneys exhibited increasing amounts of cloudy swellings, degeneration of tubular epithelia, tissue necrosis, extensive vacuolization in renal tubules, hypertrophy and atrophy of glomeruli, exudation, interstitial oedema, and interstitial nephritis. These changes in the kidneys result in impaired renal function in chronic fluoride intoxication.

Keywords: Albino rabbits, Fluorosis, Kidney pathology, Renal damage, Sodium fluoride.

INTRODUCTION

Kidneys are among the most sensitive body organs in their histopathological and functional responses to excessive amounts of fluoride.¹ They are the primary organs concerned with excretion and retention of fluoride and thus are generally involved in chronic fluoride intoxication. In humans, only a few reports²⁻⁴ pertaining to kidney involvement in endemic fluorosis are available. Kono *et al*⁶ reported impaired renal functions in fluoride-exposed workers. In contrast to cases of acute intoxication,⁵ the records of only a few autopsy reports of patients dying of chronic fluoride intoxication are traceable in the literature.^{4,7} No renal abnormality ascribed to fluoride was found at the autopsy examination of an elderly woman who for 30 years had consumed water containing 8 ppm fluoride.⁸ In three autopsy cases Reddy *et al*⁴ found multiple pyaemic abscesses in the fluorotic kidneys which grossly appeared like pyelonephritic kidneys with dilated ureters.

In rats intoxicated with 190, 210, 452, 904, and 1356 ppm fluoride in their drinking water, renal injury was reported.² Other changes included tubular degeneration, inflammation, fibrosis, parenchymatous nephritis, cloudy swellings, and dilatation of convoluted tubules.¹⁰⁻¹⁴ Shupe *et al*¹⁵ reported renal degeneration and mineralization in cattle ingesting high fluoride levels. The aim of the present study was to examine histopathological effects of sodium fluoride on the kidneys of albino rabbits.

MATERIALS AND METHODS

Animals: 60 young male and female albino rabbits weighing 400-600 g each were used. The animals were maintained on standard laboratory rabbit chow. Water was given *ad libitum*.

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Experimental design: The animals were divided equally into five groups. Doses of 5, 10, 20, and 50 mg NaF/kg bw/day in 1 mL of double distilled water were injected subcutaneously into four groups for fifteen weeks.

Control: The control animals were administered 1 mL of double distilled water/kg bw/day for the same period.

Assessment of renal damage: All the animals were killed by ether anaesthesia, and the kidneys were fixed with Carnoy's fixative and Bouin's fluid for histopathological examination. The tissue was dehydrated in tertiary butyl alcohol, cleared in amyl acetate, and embedded in paraffin. Serial sections were cut at 7 μ m and stained with iron haematoxylin and eosin.

RESULTS

On gross examination the kidneys of the fluoridated rabbits appeared shrunken as compared to those of the controls. The renal changes varied with the concentration of fluoride but were not seen in any of the control rabbits (Figure 1).



Figure 1. Photomicrograph showing the kidney of a control rabbit with normal microscopic features x 100.

In the animals treated with 5 mg NaF/kg bw/day no abnormalities in the convoluted tubules, glomeruli, and interstitial tissue were detected. Moreo-

ver, the malpighian corpuscles did not show any exudation of plasma into sub-cellular spaces.

In rabbits treated with 10 mg NaF/kg bw/day, cells lining the convoluted tubules showed cloudy swellings and dim appearance (Figure 3) as compared to the controls (Figure 1). The glomeruli exhibited hypertrophic changes (Figure 3) as compared to the controls (Figure 2). The glomerular basement membrane did not show any thickening or periglomerular fibrosis. Interstitial nephritis was well marked (Figure 4) with 4-6 large vacuoles (Figure 5).

Figure 2. Photomicrograph showing normal convoluted tubules (CT) and glomeruli (G) in the kidney of a control rabbit x 400.

In animals in the 20 mg NaF group, convoluted tubules showed cloudy swellings and extensive vacuole formation. Due to vacuolization, the cell nucleus was pushed towards the basement membrane. In some places the cell showed necrosis (Figure 6). The glomeruli were hyperatrophied and markedly lobulated (Figure 7). The glomerular membrane was slightly thickened. There was round cell infiltration in the interstitial tissue, and cell necrosis was evident in the interstitium along with interstitial oedema (Figure 8).

Figure 3. Photomicrograph of the kidney of a rabbit treated with 10 mg NaF/kg bw/day showing cloudy swellings in convoluted tubules and hyperatrophied glomeruli x 400.

Figure 4. Interstitial nephritis in the kidney of a rabbit treated with 10 mg NaF/kg bw/day x 100.

Figure 5. Photomicrograph showing formation of large vacuoles in the interstitium of the kidney of a rabbit treated with 10 mg NaF/kg bw/day x 400.

Figure 6. Convoluted tubules showing cloudy swellings, vacuolization, and cell necrosis in the kidney of a rabbit treated with 20 mg NaF/kg bw/day x 400.

Figure 7. Hyperatrophied glomeruli in the kidney of a rabbit treated with 20 mg NaF/kg bw/day x 400.

Figure 8. Interstitial oedema in the kidney of a rabbit treated with 20 mg NaF/kg bw/day x 100.

In the animals treated with 50 mg NaF/kg bw/day there was extensive cellular necrosis leading to degeneration of convoluted tubules (Figure 9). The remaining cells showed vacuolization of cytoplasm owing to which the cell nuclei were pushed to the basement membranes (Figure 10). The convoluted tubules showed cloudy swellings, tubular lumen was widened, nuclei showed disintegration, and there was exudation in the tubules. The glomeruli also exhibited atrophy and disintegration of cells, and formation of vacuoles was seen in the glomerular cells. The nuclei likewise showed disintegration, and the basement membrane was thickened in hyperatrophied glomeruli. Interstitial nephritis (Figure 11) and interstitial oedema and necrosis of cells in the interstitium (Figure 12) were most pronounced.

Figure 9. Cell necrosis in the convoluted tubules and exudation in the tubules in the kidney of a rabbit treated with 50 mg/kg bw/day x 400.

Figure 10. Atrophied glomeruli and formation of vacuoles and degeneration of nuclei in the glomeruli in the kidney of a rabbit treated with 50 mg NaF/kg bw/day x 400.

Figure 11. Interstitial nephritis in the kidney of a rabbit treated with 50 mg NaF/kg bw/day x 100.

Figure 12. Necrosis and interstitial oedema in the kidney of a rabbit treated with 50 mg NaF/kg bw/day x 400.

DISCUSSION

In renal fluoride studies there are marked discrepancies in the histological findings of the kidney which can be attributed in part to differences in the fluoride compounds used, the method of their administration, and the various species of the animals used.

During the present investigation, the kidneys of rabbits treated with 10, 20, and 50 mg NaF/kg bw/day showed cloudy swellings, interstitial oedema, and hypertrophy and atrophy of glomeruli. The appearance of cloudy swellings remained almost constant. The intensity of such swellings has been observed by others to increase with increasing dose of fluoride.^{16,17} In monkeys treated with 80 ppm fluoride in their drinking water for 16 weeks, cloudy swellings and albuminuria were reported.¹⁸ Ogilvie¹² likewise found oedema of the connective tissue between the convoluted tubules in rats ingesting 340 ppm fluoride for 100 days.

Chronic fluoride intoxication at a level of 14 mg F/kg bw/day and higher has been claimed to result in a renal lesions.^{2,10} Rats consuming 50 to 100

ppm NaF in the diet for 20 months developed dark, shrunken, and nodulated kidneys. In evidence were vascular, glomerular, and tubular degeneration leading to interstitial fibrosis. An increase in the amount of fibrous tissue in the renal lesions was also induced by chronic fluoride administration.¹⁰ Such an increase in the amount of fibrous tissue was not found during the present investigation. However, other changes seen here, like vacuolization of the cells lining the convoluted tubules, widening of lumen of tubules, hypertrophic and lobulated glomeruli, interstitial nephritis, and varying degree of degeneration of tubular epithelium, have also been detected by others.^{11,19} Shupe *et al*²⁰ found fatty degeneration and focal calcification in the stroma and tubules of the bovine kidney with bone fluoride as high as 900 ppm. Focal calcification in the tubules was not found during the present studies.

According to Kawahara,¹³ rabbits treated with 30-50 mg fluoride/kg bw/day orally for 14 to 150 days, developed inflammatory kidney changes in the glomeruli with increased cellularity, capillary hyperaemia, hypertrophy or atrophy, tubular degeneration, cloudy swellings and protein casts or blood in the lumen. In the same report, in his study on rats ingesting 5-375 ppm fluoride in drinking water for 16-234 days, he observed progressive fibrosis, round cell infiltration, changes in the glomeruli, and tubular degeneration in the groups given 100 and 375 ppm fluoride. Ramseyer *et al*²¹ also recorded hypertrophy and hyperplasia in the tubules of the rats given fluoride in the drinking water as 1, 5, and 10 ppm for 500 days. Pindborg²² observed that in rats ingesting 0.05% NaF in their diet for 21-28 days flattening of the epithelium of distal convoluted tubules and reduction in the size of the cytoplasm occurred, thereby causing the nuclei to project into the lumen. Interstitial renal oedema and inflammatory changes were also present. Kidneys of rats fed 0.05% NaF in their diet exhibited a hobnailed appearance.²³ There was extensive dilatation of the Henle loops and convoluted tubules. Focal fibrosis was responsible for the hobnailed appearance of kidneys.²⁴ However, such changes were not seen in the present study.

Taylor *et al*²⁵ noted necrosis of the tubular cells and dilatations of the tubules in the corticomedullary region in rats given 200 to 500 ppm fluoride for 5 days. Bosworth and McCay²⁶ found that rats given 1, 2, 6, and 10 ppm fluoride up to 520 days of age developed casts in the cortical and medullary region and the hyperchromatism in the convoluted tubules. The nuclei were larger in diameter and cells appeared plump. There was also accumulation of mononuclear leukocytes, and lymphocytes termed as 'Nephritis' were present in the cortex and around the blood vessels. Nephritis occurred in the interstitial tissue of the kidneys of fluoridated rabbits during the present study. Fluoride-induced hyperplasia and hypertrophy of renal tubules have also been reported in hamsters.²⁷

Kour and Singh¹⁴ observed that mice fed with 10, 500, and 1000 ppm NaF for 3 months had shrunken kidneys showing subcapsular indentation on the surface. They recorded cloudy swellings, degeneration of tubular cells, dilatation in the convoluted tubules, patchy areas of necrosis of tubular cells, atrophy of glomeruli, and interstitial infiltration of round cells. Similar changes were seen in the fluoridated rabbits during the present investigation.

Rioufol *et al*²⁸ found that guinea pigs fed with 20 ppm NaF in their diet for 12 months incurred dilatation of the proximal convoluted tubules with inflammatory infiltration in the cortical region. The glomeruli became swollen with thickening of the walls of Bowman's capsule. In the tubules cystic dilatation occurred along with hyaline cylinders.

On the other hand, some workers failed to find any gross renal change in rats and squirrel monkeys.²⁹ de Camargo and Merzel³⁰ treated rats with 1 to 100 ppm fluoride in drinking water for 180 days but did not report any abnormality with respect to weight gain, morphology, and macroscopic appearance of kidneys as compared to the controls.

From the histopathological changes seen here in kidneys in acute and chronic fluoride intoxication, it is clear that fluoride does lead to subtle renal damage which manifests itself in the form of cloudy swellings of the convoluted tubules, vacuolization and necrosis of tubules, hypertrophy and atrophy of glomeruli, interstitial oedema, and interstitial nephritis.

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