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Biochemical and histological studies on the effect of sodium fluoride on rat kidney collagen

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Abstract The present study was carried out to study the effect of acute doses of sodium fluoride on the collagen content of the rat kidneys. Five groups of rats were studied: (i) control rats and (ii) rats divided into four subgroups according to the dose of NaF. Results showed that higher doses of sodium fluoride 10, 20 and 30 mg of NaF/kg body weight caused a significant decrease in the collagen content of the kidneys when compared to the control rats. Electron microscope studies supported these results and showed the sodium fluoride doses 10, 20 and 30 mg of NaF/kg body weight caused disruption of ordered collagen fibrils of the rat kidneys.

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1. Introduction

The extracellular matrix is a complex mixture of structural and functional proteins arranged into a unique, tissue-specific three-dimensional ultrastructure. These proteins provide a natural scaffold for tissue and organ morphogenesis, maintenance, and regeneration following injury (Alberts et al., 2002). Animal connective tissues consist of mostly extracellular matrix, with collagens and elastin providing the tensile

strength to the tissues. Collagens, the most abundant proteins in the body, constitute a multigene family of extracellular matrix proteins. In addition to providing mechanical strength for various organs and tissues, they have a number of other important biological functions. The collagens represent a family of trimeric extracellular matrix molecules used by cells for structural integrity and other functions. The three alpha chains that form the triple helical part of the molecule are composed of repeating peptide triplets of glycine–X–Y. X and Y can be any amino acid but are often proline and hydroxyproline, respectively (Gordon and Hahn, 2010).

The kidneys perform a variety of functions for the body, the most important being removal of unwanted substances (waste and surplus) from the plasma, homeostasis of the body's water, electrolyte and acid/base status and participation in endocrine regulation. The amount of collagen in the kidney depends on some factors like the species of the animal, its age and the presence of disease. Nevertheless, collagen is of great physiological importance as a support for the renal parenchyma and as a component of the basement membrane (Weiss and Jayson, 1982).

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Fluoride is taken mainly in drinking water beside various nutrition products and beverages like tea, drugs, fluoride containing salt (Eren et al., 2005). Fluoride is ubiquitous in the environment; therefore, sources of drinking water are likely to contain at least some small amount of fluoride. However, in areas of the world in which endemic fluorosis of the skeleton or teeth has been well documented, the level of fluoride in drinking water supplies range from 3 to more than 20 mg/l. In areas in which drinking water is fluoridated (i.e., fluoride is intentionally added for the prevention of dental caries), the concentration of fluoride in drinking water generally ranges from 0.7 to 1.2 mg/l (WHO, 2002). Low intakes of fluoride are associated with an increased incidence of dental caries and addition of fluoride, at 1 mg/kg to water supplies reduces this. The body burden of fluoride is regulated by renal excretion. Fluorosis is excessive deposition of fluoride, particularly in the bones and teeth. It occurs when the daily intake exceeds 20 mg of fluoride (Gessner et al., 1994). Excessive fluoride ingestion has been reported to cause collagen breakdown as evidenced by the appearance of collagen breakdown products in the urine (Sharma, 1982).

The present study was carried out to evaluate the effect of sodium fluoride on the renal collagen content in rats.

2. Materials and methods

2.1. Chemicals

Chloramine-T, p-dimethylaminobenzaldehyde (Ehrlich's reagent), L-hydroxyproline, sodium acetate, citric acid, perchloric acid, *n*-propanol, sodium hydroxide, and acetic acid were purchased from Sigma Chemical Company, St. Louis, MO, USA. Double distilled water was used throughout the study.

2.2. Animal care

Healthy adult male Wistar rats weighting 150–200 g (4–6 weeks old) were obtained from Breeding Laboratory, King Saud University, Riyadh, Saudi Arabia. The animals were labeled by identifying ear notches, housed in clean cages, and placed in the animal care room. The animals were given a standard rodent chow ad libitum. The animal room was maintained at 21 ± 1.5 °C. Ethical guidelines for animal care were followed.

2.3. Animal groupings

Thirty adult male rats were divided into five equal groups. Rats of the first groups were intraperitoneally injected with the vehicle and left as control. The 2nd, 3rd, 4th and 5th groups of the rats were intraperitoneally injected with sodium fluoride in doses of 5, 10, 20, and 30 mg/kg, respectively. After 24 h the animals were sacrificed by asphyxiation with carbon dioxide.

2.4. Preparation of kidney samples for collagen determination

After the animals were killed, the kidneys were dissected out, cleared of adhering tissues, and weighed. One of the kidneys

was then homogenized in normal saline (10% w/v) and the homogenate was used for biochemical analysis.

2.5. Determination of collagen content

Total collagen content was calculated as hydroxyproline concentration assuming that hydroxyproline constitutes 12.5% of total collagen (Edwards and O'Brien, 1980).

2.6. Determination of hydroxyproline concentration

Hydroxyproline was measured by the modified alkaline hydrolysis method of Reddy and Enwemeka (1996). Briefly, to an aliquot of homogenate was added into NaOH (2 N final concentration) and the aliquot was hydrolyzed by heating in a boiling water bath for about 3–4 h. About 900 μ l of 56 mM chloramine-T reagent was added to the hydrolyzed sample and oxidation was allowed to proceed at room temperature for 25 min. Then an 1000 μ l of 1 M Ehrlich's reagent (p-dimethylaminobenzaldehyde) was added to the oxidized sample and the chromophore was developed by incubating the samples at 65 °C for 20 min. The absorbance was read at 550 nm using an Ultrospec 2000 UV/visible spectrophotometer (Pharmacia Biotech Ltd., Science Park, Cambridge, England). The hydroxyproline concentration in the samples was calculated from the standard graph of hydroxyproline.

2.7. Preparation of sample for electron microscopy

One of the kidneys was removed and fixed in 1% formalin and stored till further processed for electron microscopy. The sample was then placed in primary fixative of 2.5% glutaraldehyde overnight in refrigerator, washed with phosphate buffer pH 7.2. It was then placed in secondary fixative which was 1% osmium tetroxide over night in refrigerator and then dehydrated by series concentration of ethanol and embedded in resin mixture (SPI-Pon™ – Araldite® Epoxy Embedding Kit). This was sectioned and used for electron microscopy.

2.8. Statistical analysis

Each sample was run in duplicate. The collagen content was expressed as mean \pm SD μ g/g wet tissue, for $n = 5$ –6 rats. Kidney collagen levels between groups were compared using one-way ANOVA analysis followed by Tukey's for multiple comparison tests. Values were considered significant if $P < 0.05$. Statistical analysis was performed by means of In-Stat® package for personal computers (GraphPad™ Software, Inc., San Diego, USA).

3. Results and discussion

Though fluoride is useful in preventing dental caries, excessive intake of fluoride can be toxic. Collagen forms a small fraction of renal mass but it is important as a support for the renal parenchyma and as a component of the basement membrane. In the present study, various doses of NaF were used to study its acute toxic effects on total collagen content in rat kidneys. Fig. 1 shows the effect of different doses of NaF on total collagen content in rat kidneys. NaF at a dose of 5 mg/kg caused no significant changes in the renal collagen

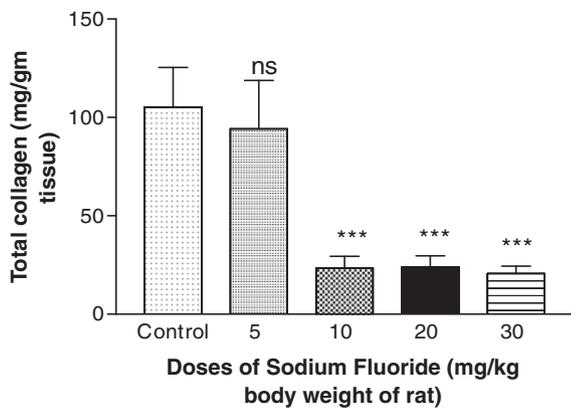


Figure 1 Effect of sodium fluoride on total collagen in rat kidneys, ns non significant; *** $P < 0.001$ as compared to control.

content of rat kidneys. These results were supported by electron microscope studies. Figs. 2 and 3 show electron micrograph in kidney of control rat and rat treated with 5 mg/kg body weight of NaF respectively. The figures show that in both groups collagen appeared as parallel fibrils. However NaF in doses of 10, 20 and 30 mg/kg body weight caused a significant decrease of 78%, 76% and 79% respectively in total collagen when compared to control group ($P < 0.001$). This may be either due to increased degradation of collagen by NaF or an inhibition of collagen synthesis by NaF (Machoy-Mokrzynska, 2004). In another explanation, Sharma (1982) stated that the mode of action of fluoride appears variable from tissue to tissue. He added that the collagen fibers produced during fluoride toxicity would be defective due to inadequate cross-links. Thus fluoride interferes with the maturation and normal metabolism of tissue collagen. The glomerulus is the functional unit of the kidney. The normal glomerular basement membrane, composed of type IV collagen, plays an important function in the process of filtration (Khubchandani et al., 2010). Therefore any alteration in the kidney collagen content is also likely to affect the renal

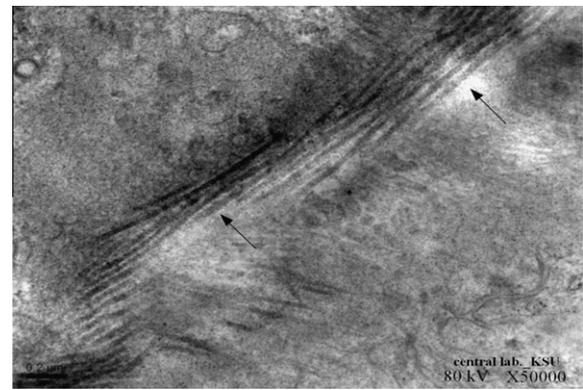


Figure 3 Electron micrograph of kidney from rat injected with 5 mg/kg body weight of NaF. Collagen appeared as paralleled fibrils in the interstitium between the cells. Indicated magnification $\times 50,000$ and total magnification 69,200.

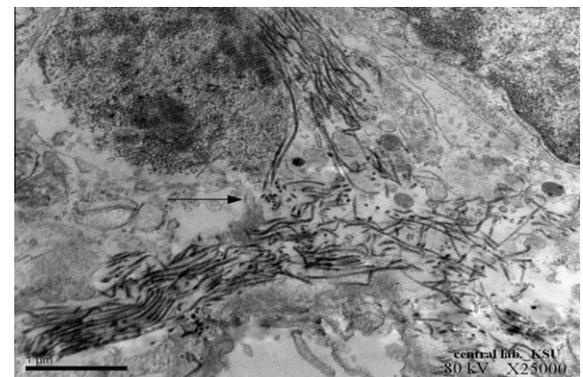


Figure 4 Electron micrograph of the kidney rat collagen injected with 10 mg/kg body weight of NaF. The characteristic parallel packing of collagen fibrils was lost completely. Indicated magnification $\times 25,000$ and total magnification 34,000.

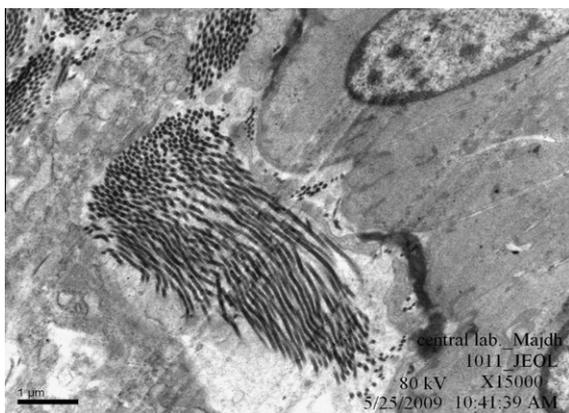


Figure 2 Electron micrograph in kidney of control animals. The collagen appeared as parallel packing of fibrils distributed in the interstitial space, glomerular mesangium, glomerular and tubular basement membrane. Indicated magnification $\times 15,000$ and total magnification 20,300.

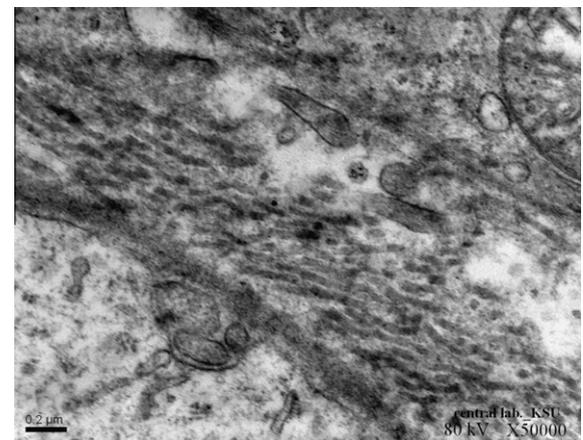


Figure 5 Electron micrograph of kidney from rat injected with 20 mg/kg body weight of NaF. The characteristic parallel packing of collagen fibrils was lost completely. They appeared as interrupted small pieces of fibrils. Indicated magnification $\times 50,000$ and total magnification 69,200.

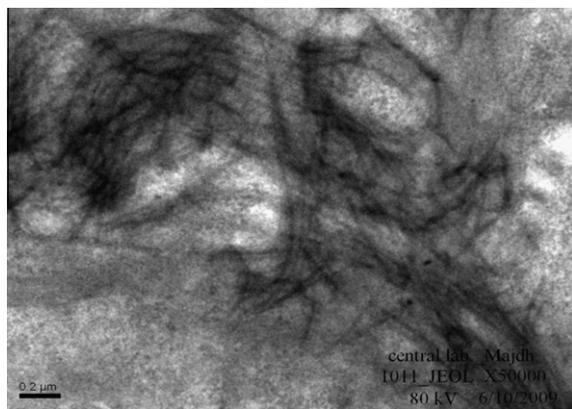


Figure 6 Electron micrograph from rat injected with 30 mg/kg body weight of NaF showing overlapping and twisting of collagen fibrils. Indicated magnification $\times 50,000$ and total magnification 69,200.

function. Our studies have demonstrated that NaF treatment of rats causes alterations in the serum biochemical parameters and electrolytes contents indicating an altered renal function (unpublished data). The electron microscope studies also demonstrated that doses of NaF 10, 20 and 30 mg/kg body weight caused alterations in the arrangement of collagen fibrils in the kidneys (Figs. 4–6). Collagen types III and VI are found in the interstitium as fine beaded fibrils (10–15 nm) and filaments (6–10 nm), respectively. Both are often found associated with thick, crossbanded type I collagen fibers (30–35 nm) and occasionally associated with some basement membranes adjacent to the interstitium.

4. Conclusion

The present study concludes that acute NaF treatment of rats caused a decrease in the renal collagen content in rats. This was accompanied by alteration in the characteristic arrangement of collagen fibers in the kidneys.

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References

- Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J., 2002. *Molecular Biology of the Cell*, fourth ed. Garland Publishing, Inc., New York. Available from: <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4&part=A3532> (accessed 01.04.10).
- Edwards, C.A., O'Brien Jr., W.D., 1980. Modified assay for determination of hydroxyproline in a tissue hydrolyzate. *Clin. Chim. Acta* 104, 161–167.
- Eren, E., Ozturk, U., Faruk, E., Canatan, D., 2005. Fluorosis and its hematological effects. *Toxicol. Ind. Health* 21, 255–258.
- Gessner, B.D., Beller, M., Middaugh, J.P., Whiteford, G.M., 1994. Acute fluoride poisoning from public water system. *N. Eng. J. Med.* 330, 95–99.
- Gordon, M.K., Hahn, R.K., 2010. *Collagens. Cell Tissue Res.* 339 (1), 247–257.
- Khubchandani, S.R., Arun, R.C., Gowrishankar, S., 2010. Banded collagen in the kidney with special reference to collagenofibrotic glomerulopathy. *Ultrastruct. Pathol.* 34 (2), 68–72.
- Machoy-Mokrzynska, A., 2004. Fluorine as a factor in premature aging. *Ann. Acad. Med. Stet.* 50 (Suppl. 1), 9–13.
- Reddy, G.K., Enwemeka, C.S., 1996. A simplified method for the analysis of hydroxyproline in biological tissues. *Clin. Biochem.* 29 (3), 225–229.
- Sharma, Y.D., 1982. Effect of sodium fluoride on collagen cross-link precursors. *Toxicol. Lett.* 10, 97–100.
- Weiss, J.B., Jayson, M.I.V., 1982. *Collagen in Health and Disease*. Churchill Livingstone, New York, pp. 404–405.
- World Health Organization, 2002. Fluorides. Available from: <http://inchem.org/documents/ehc/ehc/ehc227.htm#ref/> (accessed on 25.07.09).