

Full Length Research Paper

Studies on the comparative effect of sodium fluoride on collagen content in various rat organs

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Fluoride is an essential element for the normal development and growth of human beings. The main source of fluoride for humans is the intake of groundwater. At high levels, fluoride causes dental and skeletal fluorosis. In this study, control and sodium fluoride (NaF) treated groups of rats had significant ($p < 0.05$) higher collagen in the kidneys followed by lungs and liver. 5, 10 and 20 mg/kg body weight of NaF caused a significant decrease ($p < 0.05$) in the collagen content of kidneys, lungs and liver. Lungs had significant ($p < 0.001$) higher collagen content in magnesium ($MgCl_2$) treated group, followed by kidneys and liver. Pretreatment with $MgCl_2$ caused an increase in the collagen content of lungs and liver but not the kidneys. Though, $MgCl_2$ has been reported to be protective against NaF, it exerts an independent effect on the collagen content of tissues.

Key words: Collagen, sodium fluoride, magnesium chloride, rats.

INTRODUCTION

Collagens are trimeric molecules, composed of three polypeptide α chains, which contain the sequence repeat (G-X-Y)_n, X being frequently proline and Y hydroxyproline. These repeats allow the formation of a triple helix, which is the characteristic structural feature of the collagen superfamily. Each member of the collagen family contains at least one triple-helical domain, which is located in the extracellular matrix, and most collagens are able to form supramolecular aggregates. Besides triple-helical domains, collagens contain non triple-helical domains, used as building blocks by other extracellular matrix proteins and are thus modular proteins (Ricard-Blum and Ruggiero, 2005).

Fluoride has been described as an essential nutrient and fluorine has also been included in the list of 14 elements recognized to be physiologically essential for the normal development and growth of human beings. The fluoride ion comes from the element fluorine. Fluorine, the 17th most abundant element in the earth's crust, is a gas and never occurs in a free state in nature. Fluorine exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil (Dhar and Bhatnagar, 2009). The main source of fluoride for humans is the intake of groundwater contaminated by geological sources

(maximum concentrations reaching 30 to 50 mg/L). The level of fluoride contamination is dependent on the nature of the rocks and the occurrence of fluoride-bearing minerals in groundwater. At high levels, it is known to cause dental and skeletal fluorosis. Very high levels of fluoride affect various body organs and genetic material. This study was undertaken to compare the effect of various doses of sodium fluoride on collagen content in different rat organs.

MATERIALS AND METHODS

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 Co. St Louis, MO, USA. Double distilled water was used throughout this study.

Animal care

Healthy adult male Wistar rats weighing 150 to 200 g (four to six weeks old) were obtained from Breeding Laboratory, King Saud University, Riyadh, Saudi Arabia. The animals were labeled by identifying ear notches. They were housed in clean cages, and

placed in the animal care room. Ethical guidelines for animal care were followed.

The rats were divided into four equal groups each consisting of 4 to 6 rats. Rats of the first group served as control. The 2nd, 3rd and 4th groups of the rats were injected intraperitoneally (ip) with sodium fluoride in doses of 5, 10 and 20 mg/kg body weight, respectively. After 24 h, the animals were sacrificed by asphyxiation with carbon dioxide.

To study the protective effect of $MgCl_2$, the following groups of rats were studied (1) normal rats (Control group, $n = 4$ to 6 rats); (2) rats injected with $MgCl_2$ through intraperitoneal route 30 mg/kg body weight ($MgCl_2$ treated group); (3) rats injected with NaF through intraperitoneal route 10 mg/kg body weight (NaF treated group); (4) rats injected with $MgCl_2$ through intraperitoneal route 30 mg/kg body weight followed by NaF 10 mg/kg body weight. The animals were sacrificed 24 h after $MgCl_2$ injection.

Preparation of the samples

After the animals were killed, the kidneys, lungs and liver were dissected out, cleared of adhering tissues, and weighed. The organs were homogenized in normal saline (10% w/v) and the homogenate was used for the determination of collagen concentration.

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).

Determination of hydroxyproline concentration

Hydroxyproline was measured by the modified alkaline hydrolysis method of Reddy and Enwemeka (1996). Briefly, 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 to 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, 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.

Statistical analysis

Each sample was run in duplicate. The collagen content was expressed as mean \pm SD mg/g wet tissue, for $n = 5$ to 6 rats. 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).

RESULTS AND DISCUSSION

It is well established that prolonged use of fluoride at recommended levels does not produce any harmful phy-

siological effects in human. However, there are safe limits for fluoride beyond which harmful effects can occur. These effects can be classified as acute and chronic toxicity (Dhar and Bhatnagar, 2009). Symptoms of acute toxicity occur rapidly. They include abdominal pain, diarrhea, vomiting, excess salivation and thirst (Dhar and Bhatnagar, 2009). The liver and kidney are the target organs markedly attacked by excessive amount of fluoride. High doses of fluoride intake lead to changes of structure, function, and metabolism in liver and kidney (Yang and Liang, 2011). In this study, NaF treatment caused significant changes in the collagen content of liver, lungs and kidney.

Figure 1 shows the effect of different doses of NaF on the collagen concentration in three different rat tissues viz., liver, lungs and kidneys. In all the groups studied, kidney had the highest collagen concentration followed by lungs and liver. There existed a significant ($p < 0.001$) difference in the collagen concentration of the lungs and liver when compared to kidneys in the control and the group treated with 5 mg/body weight NaF. 10 and 20 mg/kg body weight NaF caused a significant decrease in the collagen content of liver when compared to kidneys ($p < 0.001$). However, 10 and 20 mg/kg body weight NaF did not cause any significant change in the lung collagen when compared to kidneys of the same group ($p > 0.05$). 10 and 20 mg/kg body weight NaF caused a significant decrease in the collagen concentration of kidneys when compared to the control and 5 mg/kg body weight NaF treated group. 10 and 20 mg/kg body weight NaF caused an increase in collagen content of lungs. This may be due to the fact that fluoride has been reported to increase the protein concentration in lungs (Stawiarska-Pieta et al., 2009). Fluoride exerts diverse cellular effects in a time-, concentration-, and cell-type-dependent manner. The main toxic effect of fluoride in cells consists of its interaction with enzymes. In most cases, fluoride acts as an enzyme inhibitor, but, fluoride ions can occasionally stimulate enzyme activity. The mechanisms depend on the type of enzyme that is affected (Adamek et al., 2005). Fluoride at micromolar levels is considered an effective anabolic agent because it promotes cell proliferation, whereas millimolar concentrations inhibit several enzymes, including phosphatases, both *in vivo* and *in vitro* (Mendoza-Schulz, 2009).

In our previous studies, we demonstrated that NaF at concentration of 20 and 30 mg/kg body weight caused a significant decrease in the activity of serum alkaline phosphatase (Siddiqi et al., 2011).

Several observations suggest that the magnesium content of a diet may influence the food fluoride absorption. Magnesium and fluoride form of an insoluble complex *in vitro*. Simultaneous administration of magnesium and fluoride by gastric intubation has been shown to significantly reduce skeletal uptake of fluoride by growing rats, which may explain why high dietary magnesium appears to ameliorate fluorosis in guinea pigs (Cerlewski,

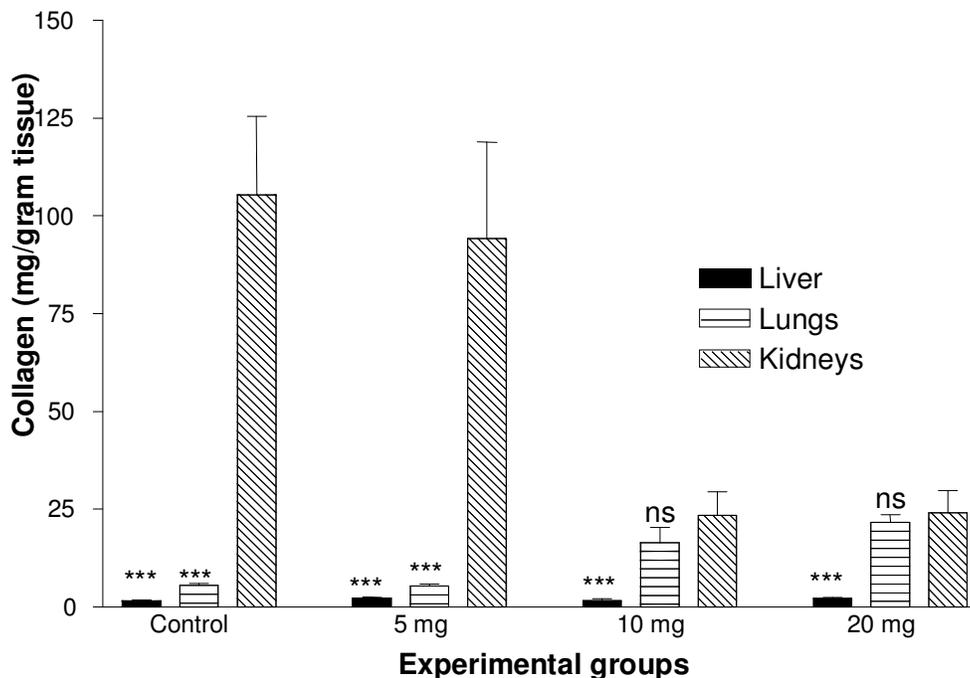


Figure 1. Effect of various doses of sodium fluoride on collagen content in different rat organs. *** P < 0.001 when compared to kidneys in the same group. Tukey's multiple comparison test. ns non significant when compared to kidneys in the same group. Tukey's multiple comparison test.

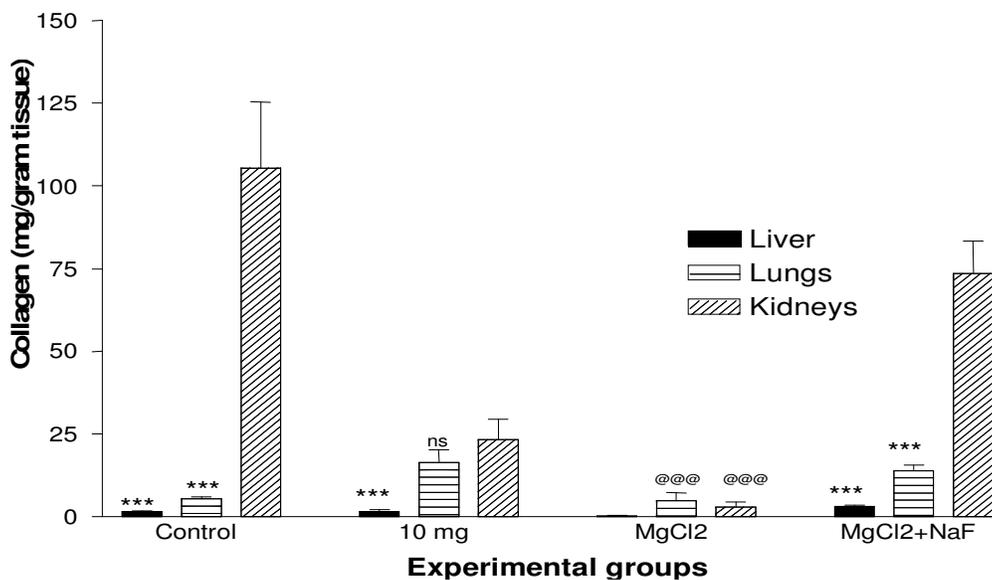


Figure 2. Effect of magnesium chloride and sodium fluoride on collagen content in different organs. *** P < 0.001 when compared to kidneys in the same group. Tukey's multiple comparison test. @@@ P < 0.001 when compared to lungs in the same group. Tukey's multiple comparison test.

1987). Magnesium chloride administered thirty minutes before sodium fluoride has been shown to increase the LD50 for fluoride from 76 to 104 mg/kg body weight (Luoma et al., 1984).

Figure 2 shows the effect of pretreatment of MgCl₂ followed by NaF treatment on collagen concentration in various rat organs. Lungs had the highest collagen content in MgCl₂ treated group followed by kidneys and liver. The

difference between the collagen content of lungs and liver was significant ($p < 0.001$). The administration of $MgCl_2$ before NaF caused a significant increase in collagen content in kidneys, lungs and liver. This may be due to the fact that magnesium forms complex with fluoride and decreases its absorption from the intestine (Barbier et al., 2010). In $MgCl_2$ plus NaF treated group, kidneys had significantly higher collagen concentration followed by lungs and liver.

Conclusion

The result of this present study suggests that though $MgCl_2$ has been reported to ameliorate sodium fluoride induced toxicity, it also has an independent effect on collagen content of various rat organs.

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