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# PROTECTIVE EFFECT OF MAGNESIUM CHLORIDE ON SODIUM FLUORIDE INDUCED ALTERATIONS IN VARIOUS HYDROXYPROLINE FRACTIONS IN RAT LUNGS

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**Abstract**

Frequent absorption of the fluoride causes tooth decay, damage of kidneys, bones, nerves and muscles. The present study was carried out to study the reported protective effect of magnesium chloride on sodium fluoride (NaF) induced alterations in rat lung hydroxyproline/ collagen content. To study the dose response of NaF following groups were studied: (i) normal rats (ii) placebo group, (iii) rats treated with two different doses of NaF. To study the protective effect of $MgCl_2$ the following groups of rats were studied (i) normal rats (ii) rats injected with $MgCl_2$ (iii) rats injected with NaF (iv) rats injected with $MgCl_2$ followed by NaF. Sodium fluoride doses of 10 and 20 mg/kg body weight of rats caused a significant increase ($p < 0.001$) increase in peptide - bound and total Hyp content in rat lungs. Administration of $MgCl_2$ alone to rats also caused significant increase in peptide- bound, protein- bound and total Hyp fractions in rat lungs ($p < 0.001$). Administration of $MgCl_2$ thirty minutes before NaF restored the altered protein bound Hyp fraction to almost normal levels. The present study concludes that although $MgCl_2$ has been reported to be protective against toxic effects of NaF, it exerts an independent effect on hydroxyproline and collagen content in rat lungs.

***Key words:*** Collagen, hydroxyproline, sodium fluoride, magnesium chloride, rats, lungs.

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**Abbreviations:** Hyp : Hydroxyproline.

## INTRODUCTION

Fluoride occurs in environment in combination with other elements, as a fluoride compounds (14). Besides food (9), inhalation (10) is another route of fluoride exposure. Excessive exposure to fluoride leads to acute poisoning, cerebral edema, and degeneration of the liver and kidneys. Chronic (8) and acute intoxication through the airways produces coughing, choking, and chills, followed by fever and pulmonary edema. Concentrated solutions of fluorine compounds produce necrotic lesions which are difficult to heal (13). The adverse toxic effects of fluoride arise due to a) enzyme inhibition b) collagen break down c) gastric damage and d) disruption of the immune system (3). In spite of its toxic effects, fluoride continues to be used in different products. Fluorine compounds have been shown to act on organic part of supporting tissues including collagen and cells of the connective tissue (13).

 

Collagen is an integral component of lung. It maintains alveolar, airway, and vascular stability, limits lung expansion, and contributes significantly to lung recoil at all lung volumes (7). One of the most commonly used marker of collagen breakdown/ degradation is hydroxyproline. The hydroxyproline (Hyp) is a post translational product of proline hydroxylation catalyzed by the enzyme prolylhydroxylase (EC 1.14.11.2) (17). The occurrence of this amino acid is thought to be confined exclusively to collagen, where it is present in the Y position of the Gly-X-Y repeating tripeptide (15). Consequently, the presence of hydroxyproline in tissues can be used as a measure of collagen or collagen degradation products (18).

The present study was carried out to study the reported protective effect of magnesium chloride ($MgCl_2$) on sodium fluoride (NaF) induced alterations on various Hyp fractions in rat.

## MATERIALS AND METHODS

### *Chemicals*

Chloramine-T, p-dimethylaminobenzaldeyde (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 the study.

### *Animal Care*

Healthy adult male Wister rats weighting 150-200 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, housed in clean cages, and placed in the animal care room. Ethical guidelines for animal care were followed.

Rats were allowed free access to food (Purina rodent chow) and tap water for one week. After one week, rats were divided into different groups. To study the dose response of rats, the following groups were studied: (i) normal rats (Control group, n = 4 - 6 rats); (ii) placebo group rats were injected with normal saline intraperitoneally (ip). Rats were divided into two subgroups according to the dose of NaF viz., rats were injected with a single dose ip of (iii) a) 10, and b) 20 mg of NaF/kg body weight/24 hours (sodium fluoride treated group, n = 5-6 rats).

To study the protective effect of $MgCl_2$the following groups of rats were studied (i) normal rats (Control group, n = 4 - 6 rats); (ii) rats injected with $MgCl_2$ through intraperitoneal route 30 mg/kg body weight ($MgCl_2$ treated group); (iii) rats injected with NaF through intraperitoneal route 10 mg/kg body weight (NaF treated group); (iv) rats injected with $MgCl_2$ through intraperitoneal route 30 mg/kg body weight followed by NaF 10 mg/kg body weight through intraperitoneal route 30 minutes after $MgCl_2$ injection ($MgCl_2$ + NaF treated group).

### *Preparation of the sample*

The animals were killed 24 hours after the NaF injection by carbon dioxide asphyxiation. The lungs were dissected out, cleared of adhering tissues and weighed. The lungs were then homogenized in normal saline (10% W/V) and the homogenate was used for Hyp determination as described below.

### *Extraction of Free, Peptide- and Protein-bound Hydroxyproline*

Free and protein-bound Hyp was extracted by the method of Varghese *et al.*, 1981 (20) with slight modification. Briefly, 0.5 mL of the homogenate was treated with 3 X 2 ml portion of re-rectified absolute alcohol and centrifuged at 600 g for 10 min. The supernatants were pooled and evaporated to dryness. The residue was dissolved in suitable amount of distilled water and an aliquot of the extract was used for estimation of free Hyp. The peptide-bound Hyp was determined after alkaline hydrolysis of the ethanol extractable fraction. The pellets were dissolved in distilled water and an aliquot of the extract was used for determination of protein-bound Hyp. The precipitate obtained on ethanol treatment of the homogenate was subjected to alkali hydrolysis to determine protein-bound Hyp. Further details about the extraction of Hyp fractions were described previously (19).

### *Determination of Hydroxyproline Concentration*

Hyp was measured by the modified alkaline hydrolysis method of Reddy and Enwemeka, 1996 (18). Briefly, to an aliquot of the sample 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. An aliquot 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 aliquot of 1 M Ehrlich's reagent (p-dimethylaminobenzaldehyde) was added to the oxidized sample and the chromophore was developed by incubating the samples at $65^0$C for 20 min. The absorbance was read at 550 nm using an Ultrospec 2000 UV/visible spectrophotometer (Pharmcia Biotech Ltd, Science Park, Cambridge, England). The Hyp concentration in the samples was calculated from the standard curve of Hyp. Further details about the optimization, linearity, specificity, precision and reproducibility of the method were described previously (19).

### *Statistical Analysis*

Each sample was run in duplicate. The Hyp content was expressed as mean ± SD µg/gram wet tissue, for n = 5 rats. Lung Hyp levels between groups were compared using one way ANOVA analysis followed by Tukey's for multiple comparison test. Values were considered significant if $P < 0.05$. Statistical analysis was performed by means of InStat® package for personal computers (GraphPad ™ Software, Inc., San Diego, USA).

## RESULTS

Table 1 shows the effect of different doses of NaF on different Hyp fractions in rat lungs.

 

All the doses of NaF used caused no significant ($P > 0.05$) change in the free and protein – bound Hyp in rat lungs. 10 and 20 mg/kg body weight dose of NaF caused a significant increase in peptide-bound Hyp ($P < 0.001$) when compared to control group. Doses of 10 and 20 mg/kg body weight dose NaF also caused a significant increase in total Hyp by 200% and 300% respectively ($p < 0.001$) when compared to control group.

Figure 1 shows the effect of different doses of NaF on total collagen in rat lungs. The change in total collagen content in rat lung was parallel to that of total Hyp concentration. Only NaF doses of 10 and 20 mg/kg body weight caused an increase of 200% and 300% ($p < 0.001$) respectively in total lung collagen when compared to control rats.

**Table 1.** Effect of different doses of sodium fluoride on various hydroxyproline fractions in rat lungs

| Experimental Groups | Free Hyp (µg/gm fresh tissue) | Peptide-bound Hyp (µg/gm fresh tissue) | Protein-bound Hyp (µg/gm fresh tissue) | Total Hyp (µg/gm fresh tissue) |
|---|---|---|---|---|
| Control | 77.17 ± 7.6 | 11.67 ± 1.2 | 597.3 ± 64.75 | 686.1 ± 69.35 |
| Placebo | 72.99 ± 2.2 $^{ns}$ | 13.72 ± 1.1 $^{ns}$ | 542.7 ± 130.5 $^{ns}$ | 629.4 ± 130.2 |
| 10 mg/kg body weight of NaF | 50.70 ± 6.7 $^{ns}$ | 1003 ± 400*** | 1004 ± 277.8 $^{ns}$ | 2061± 473.8*** |
| 20 mg/kg body weight of NaF | 110.6 ± 45.5 $^{ns}$ | 1695 ± 174.8*** | 938.5 ± 122.6 $^{ns}$ | 2744 ± 241.4*** |

Values are expressed as mean ± SD kidney weight (4- 6 rats/group)
$^{ns}$ non significant as compared to control group (Tukey's multiple comparision test);
***P < 0.001 compared to control group (Tukey's multiple comparision test).
Animals were injected with sodium fluoride through intraperitoneal route. Rats were sacrificed 24 hours after the treatment.

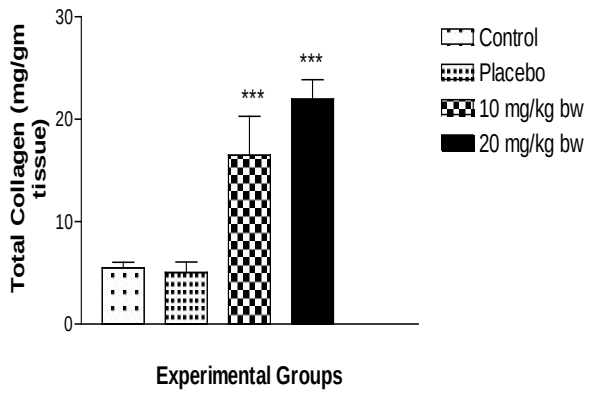


**Figure 1.** Effect of different doses of NaF on total collagen in rat lungs. Rats were given sodium fluoride through intraperitoneal route. The animals were sacrificed 24 hours after the treatment. Values are expressed as mean ± SD ( 4-6 rats/group). $^{ns}$ non significant as compared to control group (Tukey's multiple comparision test); ***P < 0.001 compared to control group (Tukey's multiple comparision test).

Table 2 shows the effect of $MgCl_2$ and NaF on different Hyp fractions in rat lungs. $MgCl_2$ treatment alone caused a significant increase in peptide- bound, protein- bound and total Hyp content of lungs when compared to control rats. Administration of $MgCl_2$ thirty minutes before NaF injection restored the protein bound- Hyp to almost normal levels but failed to restore peptide-bound and total Hyp to normal levels.

Figure 2 shows the effect of $MgCl_2$ and NaF on total collagen in rat lungs. $MgCl_2$ treatment caused a significant increase in total lung collagen ($p < 0.001$) when compared to control rats. Similarly NaF alone also caused a significant increase in total collagen ($p < 0.001$) when compared to control rats. $MgCl_2$ treatment before NaF administration caused a decrease in total collagen which was significant ($p < 0.001$) when compared to $MgCl_2$ treated group but not significant to NaF alone treated group ($p < 0.5$).

 

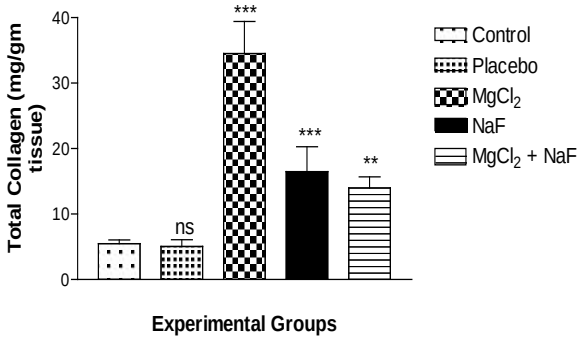


**Figure 2.** Effect of $MgCl_2$ + NaF on total collagen in rat lungs. Rats were given sodium fluoride and $MgCl_2$ through intraperitoneal route. The animals were sacrificed 24 hours after the treatment. Values are expressed as mean ± SD (4-6 rats/group). $^{ns}$ non significant as compared to control group (Tukey's multiple comparision test); **P < 0.01 compared to control group (Tukey's multiple comparision test). ***P < 0.001 compared to control group (Tukey's multiple comparison test).

**Table 2.** Effect of magnesium chloride and sodium fluoride on various hydroxyproline fractions in rat lungs

| Experimental Groups | Free Hyp (µg/gm fresh tissue) | Peptide-bound Hyp (µg/gm fresh tissue) | Protein-bound Hyp (µg/gm fresh tissue) | Total Hyp (µg/gm fresh tissue) |
|---|---|---|---|---|
| Control | 77.17 ± 7.6 | 11.67 ± 1.2 | 597.3 ± 64.75 | 686.1 ± 69.35 |
| Placebo | 72.99 ± 2.2 $^{ns}$ | 13.72 ± 1.1 $^{ns}$ | 542.7 ± 130.5 $^{ns}$ | 629.4 ± 130.2 |
| $MgCl_2$ | 51.85 ± 10.66 $^{ns}$ | 2687 ± 132.3*** | 1575 ± 493.3*** | 4314 ±608.4*** |
| NaF | 50.70 ± 6.7 $^{ns}$ | 1003 ± 400*** | 1004 ± 277.8 $^{ns}$ | 2061± 473.8*** |
| $MgCl_2$ + NaF | 40.45 ± 6.4 $^{ns}$ | 1295 ± 183.8*** | 411.0 ± 70.72 $^{ns}$ | 1714 ± 212.4** |

Values are expressed as mean ± SD kidney weight ( 4- 6 rats/group)
$^{ns}$ non significant as compared to control group (Tukey's multiple comparision test);
***P < 0.001 compared to control group (Tukey's multiple comparision test).
Animals were injected with $MgCl_2$ (30 mg/kg body weight) through intraperitoneal route. Animals were injected with sodium fluoride (10 mg/kg body weight) 30 minutes after $MgCl_2$ injection through intraperitoneal route. Rats were sacrificed 24 hours after NaF the treatment.

## DISCUSSION

The main route of fluoride exposure is through ingestion although other routes like inhalation are also common in many work places. Fluorine compounds also act on the organic part of supporting tissues, including collagen and other proteins, and on cells of the connective tissue. These interactions reduce the content of collagen proteins, modify the structure and regularity of collagen fibers and induce mineralization of collagen (13).

Collagen is an integral component of the lung. The collagen in the lungs function to help in gas exchange and therefore maintain the elasticity of the lung tissue. In lungs collagen is found throughout the extracellular space, alveolar interstitium, endothelial and basement membrane. The collagen in the lungs may also be contributed from the surfactant protein A of the alveolus (11). The changes in the collagen function may contribute to changes in lung function. In the present study the doses of NaF used caused no significant changes in free and protein bound Hyp content in the lungs. These fractions seemed to be resistant to changes induced by NaF. This may suggest that the doses of NaF used did not cause the breakdown of collagen because one of the repository of free Hyp is the breakdown of mature collagen (1). The fraction of Hyp affected was peptide- bound Hyp which was significantly increased by 10 and 20 mg/kg body weight of NaF. This may be the initial response to NaF assault by the lungs. Similarly the increase in total Hyp and total collagen content in the lungs by 10 and 20 mg/kg

 

body dose of NaF was due to increase mainly contributed by peptide bound Hyp fraction which may suggest that NaF stimulates fibrosis in lungs. In our earlier studies (4) we have shown that protein bound Hyp fraction of the kidneys was the least susceptible to the effect of different doses of Hyp which the other fractions viz., free, peptide bound and total Hyp showed considerable decrease with increasing doses of NaF when compared to control rats. Magnesium chloride alone also caused a significant increase in peptide bound, protein bound and total Hyp suggesting that it independently exerts it effects the Hyp and collagen content in rat lungs. Magnesium chloride administered thirty minutes before sodium fluoride has been shown to increase the $LD_{50}$ for fluoride from 76 to 104 mg/kg body weight (12). Administration of magnesium chloride before sodium fluoride restored the protein- bound Hyp to near levels which was altered by magnesium chloride alone. However peptide -bound Hyp, total Hyp and total collagen were not restored to near normal levels by magnesium chloride administered before sodium fluoride. The results of the present study suggest that though magnesium chloride may be protective against sodium fluoride induced toxicity, it exerts independent effect on collagen and hydroxyproline fractions in the lungs. The increase in the peptide and protein bound hydroxyproline content of the lungs by magnesium chloride could be due to stimulation of collagenolysis by magnesium chloride. Collagen is degraded by three mechanisms viz., by the enzyme collagenase, by phagocytosis and degradation of newly synthesized collagen by an independent mechanism (6). It is possible that magnesium chloride stimulated one or more of these processes. Increased total Hyp by magnesium chloride may also indicate the development of fibrosis in the lungs (16, 21). Increased lung total Hyp has been shown to be associated with increased collagen breakdown following lung injury (2).

The increased peptide- bound Hyp concentration in the lungs after $MgCl_2$ and NaF treatment may also be the proteolytic product of a much larger glycoprotein molecule that, like basement-membrane glycoproteins which contain hydroxylysine and hydroxylysine- linked carbohydrate. In contrast with collagens and procollagens, the alveolar glycoprotein is characterized by alternating short collagenous and noncollagenous segments, as opposed to a long collagenous peptide that is either preceded or followed by non-collagenous regions (5).

The present study therefore concludes that though $MgCl_2$ has been reported to be protective against the deleterious effects of NaF, it may also exert an independent effect on the Hyp/collagen content of the lungs.

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Other articles in this theme issue include references (22-37).

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