EFFECT OF FLUORIDE AND MAGNESIUM ON IN VIVO ACTIVITY OF INDOLEAMINE 2,3-DIOXYGENASE IN RAT LUNGS

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SUMMARY: The physiological significance of the enzyme indoleamine 2,3-dioxygenase (IDO) (EC 1.13.11.17), which consumes superoxide anion (O$_2^-$), is not clear. Since IDO is found in high concentration in lung tissue, the possibility that it may protect against oxidative stress induced in the lung by fluoride (F, as NaF) was studied in vivo in rats. To determine the dosage response to NaF, the IDO activity in the lungs was examined in the following five groups of adult Wistar male rats, each consisting of six rats: (i) normal (control) group; (ii) placebo group injected intraperitoneally (ip) with 0.5 mL saline; and (iii) three subgroups given single ip injections of 5, 10, and 20 mg NaF/kg bw. The 10 mg NaF/kg bw dosage caused a significant increase (p<0.001) in pulmonary IDO activity compared to that of the control group. On the other hand, the 5 mg NaF/kg bw injection resulted in a significant decrease (p<0.01) in IDO activity, whereas the 20 mg NaF/kg bw injection left the IDO activity essentially unchanged. To study the expected protective effect of MgCl$_2$ on the NaF-induced changes in lung IDO activity, the IDO activity in the lungs of the following four groups of male rats with six rats in each group was determined: (i) a second control group; (ii) a group injected ip with 30 mg MgCl$_2$/kg bw; (iii) a group injected with 10 mg NaF/kg bw; and (iv) a group injected with 30 mg MgCl$_2$/kg bw followed 30 min later with 10 mg NaF/kg bw. The MgCl$_2$ injection also caused a significant increase (p<0.001) in pulmonary IDO activity compared to the control group. However ip injection of MgCl$_2$ 30 min before the NaF injection did not cause any significant increase or decrease in pulmonary IDO activity.

Keywords: Fluoride and lungs; Indoleamine 2,3-dioxygenase; Magnesium chloride; Rat lungs.

INTRODUCTION

Indoleamine 2,3-dioxygenase (IDO) is a cytosolic enzyme that catalyzes oxidative cleavage of the indole ring in L-tryptophan to N-formylkynurenine, which, in turn, decomposes spontaneously into formate and L-kynurenine. The latter is then metabolized further in the kynurenine pathway.1,2 There is increasing evidence that, in addition to defense against pathogens, IDO exerts important immunomodulatory effects. In this connection, it is potentially relevant that IDO uses superoxide anion radical (O$_2^-$) as a substrate and a cofactor in its catalytic process.3, 4

On consumption of O$_2^-$ IDO initiates the formation of tryptophan metabolites, including 3-hydroxyanthranilic acid and 3-hydroxykynurenine, which are potent free radical scavengers.2 The capability of transferring a pro-oxidant (O$_2^-$) into antioxidants makes IDO a powerful modulator of oxidative stress.2,5 Fluoride (F) at various doses has been shown to induce lipid peroxidation and changes in drug metabolizing enzymes.6 The present in vivo study was therefore conducted to study the effect of different doses of sodium fluoride (NaF) on IDO activity in rat lungs. In addition, the known protective effect of magnesium on F-induced changes on IDO pulmonary activity was examined.7

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MATERIALS AND METHODS

Chemicals: All reagents and chemicals, including ascorbic acid, bovine serum albumin, catalase, p-dimethaminobenzaldehyde (Ehrlich’s reagent), kynurenine, methylene blue, potassium dihydrogen phosphate, trichloroacetic acid, and L-tryptophan, were purchased from Sigma Chemical Company, St Louis, MO, USA. Double distilled water was used throughout the study.

Animal Care: Healthy 4-to-5-week-old male Wistar rats weighing 150–200 g were obtained from the Animal Breeding Laboratory, King Saud University, Riyadh, Saudi Arabia. Since the rats were kept in an animal care room, which is an extension of the breeding laboratory, the animals were acclimatized for one day before starting the treatment. The rats were then divided into different groups of six rats each as described below. The experimental protocol was approved by the King Saud University Research Center, and the ethical animal care guidelines were followed.

Dose-response of NaF on indoleamine 2,3-dioxygenase activity in rat lungs: For these experiments, five groups of six rats each were studied: (i) normal (control) rats; (ii) placebo rats injected with 0.5 mL intraperitoneal saline; (iii) F-treated rats administered single ip injections of 5, 10, and 20 mg NaF/kg bw, respectively. After 24 hr the animals were sacrificed by asphyxiation with carbon dioxide.

Protective effect of MgCl2 on NaF-induced alteration in indoleamine 2,3-dioxygenase activity in rat lungs: The following four groups of six rats each were studied: (i) another group of normal (control) rats; (ii) rats injected ip with 30 mg MgCl2/kg bw; (iii) rats injected ip with 10 mg NaF/kg bw; and (iv) rats injected ip with 30 mg MgCl2/kg bw followed 30 min later with ip injection of 10 mg NaF/kg bw. After 24 hr these rats were likewise sacrificed by asphyxiation with carbon dioxide.

Preparation of lung samples: After the animals were killed, the lungs were dissected out, cleared of adhering tissues, and weighed. The lungs were then homogenized in normal saline (10% w/v). The homogenate was centrifuged at 13,000 rpm for 60 min and used for determination of IDO activity.

Measurement of indoleamine 2,3-dioxygenase activity: The activity of indoleamine 2,3-dioxygenase in the lung extract was measured by the method Kudo and Boyd8 with slight modifications. Briefly, 0.4 mL of enzyme extract was added to the buffer mixture. The buffer mixture consisted of 0.1 mL of 10 mM L-tryptophan, 0.2 mL of 200 µM of methylene blue, 0.2 mL of 400 mM ascorbic acid, 77 µL of suitably diluted catalase which had an enzyme activity of 200 units/mL (from stock of 518,700 units/mL), and 0.5 mL of 0.4 M pH 6.5 potassium phosphate buffer. Before mixing, the enzyme supernatant and the incubation buffer mixture were pre-heated to 37ºC. The final volume of the reaction mixture was made up to 2 mL with double distilled water. The mixture was incubated for 20 min at 37ºC, and 0.2 mL of 30% (w/v) trichloroacetic acid was added to stop the reaction. The mixture was heated at 50ºC for 30 min to hydrolyze the N-formylkynurenine formed from L-kynurenine released by the action of
indoleamine 2,3-dioxygenase on the L-tryptophan. The mixture was then centrifuged at 6,000 rpm at room temperature to remove sediment. To 1.0 mL of the supernatant was added 1.0 mL of 1% (w/v) p-dimethylaminobenzaldehyde in acetic acid. The absorbance was determined at 480 nm to measure the amount of L-kynurenine by a standard intensity calibration. The enzyme activity was expressed as mean ±SD×10⁻³ micrograms of L-kynurenine liberated/min/mg protein. Protein concentration was determined by the method of Markwell et al. using bovine serum albumin as standard.

Statistical Analysis: Samples were run in duplicate. IDO activity between groups was compared using one-way ANOVA analysis followed by Tukey’s test for multiple comparison. Values were considered significant at p<0.05. Statistical analysis was performed by means of InStat® package for personal computers (GraphPad TM Software, Inc., San Diego, USA).

RESULTS

Table 1 shows the effect of three different dosages of NaF on rat lung IDO activity.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>IDO activity⁴</th>
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<tbody>
<tr>
<td>Control</td>
<td>4.47 ± 1.15</td>
</tr>
<tr>
<td>Placebo</td>
<td>3.57 ± 0.48</td>
</tr>
<tr>
<td>NaF (5 mg/kg bw)</td>
<td>2.52 ± 0.25</td>
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<tr>
<td>NaF (10 mg/kg bw)</td>
<td>8.53 ± 1.11</td>
</tr>
<tr>
<td>NaF (20 mg/kg bw)</td>
<td>3.75 ± 0.85</td>
</tr>
</tbody>
</table>

⁴Enzyme activity is expressed as ± SD×10⁻³ micrograms of L-kynurinine formed/min/mg protein.
⁵Nonsignificant when compared to control group (Tukey’s multiple comparison test).
*p<0.01 compared to control group (Tukey’s multiple comparison test).
†p<0.001 compared to control group (Tukey’s multiple comparison test).

A 5 mg/kg bw ip injection of NaF caused a significant decrease (p<0.01) in IDO activity compared to that of the control rats, whereas a 10 mg/kg bw injection resulted in an even more significant increase (p<0.001) in IDO activity. On the other hand, 20 mg/kg bw caused no significant change (p>0.05) in pulmonary IDO activity.

Table 2 shows the protective effect of ip injection of MgCl₂ (30 mg/kg bw) on changes induced by NaF (10 mg/kg bw) in rat lung IDO activity. MgCl₂ and NaF injections alone incurred significant increases (p<0.001) in IDO activity, but administration of MgCl₂ 30 min before injection of NaF resulted in return of IDO activity in the lungs to near normal levels.
DISCUSSION

The first noticeable sign of excessive exposure to and ingestion of F in animals and humans is discoloration of dental enamel. Abnormalities in mineralization processes affect the osteoarticular system and are associated with changes in the density and structure of the bone along with irregular mineralization of osteoid tissue.

As is well known, F emissions often occur as airborne pollutants during the production of aluminum, ceramics, glass, brick products, and phosphate fertilizers. Rats exposed to F aerosols have decreased lung weight and increased activity of lactate dehydrogenase and altered protein content in bronchioalveolar lavage. The same study also demonstrated that rats exposed to NaF had an increased production of superoxide anions by the neutrophils. Since IDO uses superoxide anion radical ($O_2^-$) as a substrate and a cofactor in its catalytic process, the present study was carried out to study the effect of various dosages of NaF on rat pulmonary IDO activity and the protective effect of MgCl$_2$ on NaF-induced changes on that activity. IDO is found in high concentration in lungs and may play a role in oxidative stress induced by F. In the present study, ip injection of 5 mg and 10 mg NaF/kg bw caused significant changes in IDO activity, but injection with 30 mg MgCl$_2$/kg bw followed by 10 mg NaF/kg bw did not. These results suggest that NaF induced only transient changes in lung IDO activity. An earlier study has shown that although bacterial LPS (lipopolysaccharide) caused a four-fold increase in rat pulmonary IDO activity, it failed to protect against paraquat-induced pulmonary toxicity. The authors of that study also found that only in the rabbit is IDO able to scavenge superoxide anions and act as a protective enzyme against oxidative stress. The present study also concludes that IDO may not act as a protective agent against superoxide anions in rat lungs.

Magnesium chloride has been shown to protect against NaF as early as 1954. Later studies showed that nephrocalcitonic effect of NaF could be prevented by administering a three-fold ratio of Mg to F 30 min prior to exposure to F. In the present study a dose of 30 mg MgCl$_2$/kg bw resulted in a nearly 1.5-fold increase in lung IDO activity indicating that MgCl$_2$ is also an inducer of IDO activity. However, administration of MgCl$_2$ before NaF caused the lung IDO activity to
return to near normal levels. Studies of Comai et al.\textsuperscript{14} have also shown that there is an age-related decline in the activity of rat lung IDO.

CONCLUSION

Rat lung IDO activity is increased by NaF and MgCl\textsubscript{2} separately, but when administered together in sequence they do not increase IDO activity in the lungs.

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REFERENCES