EFFECT OF FLUORIDE AND A STANDARDIZED POLLEN EXTRACT ON THE HEPATIC P-450 SYSTEM IN RATS

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SUMMARY: The influence of a standardized pollen extract (Cernitin) on the hepatic cytochrome P-450 system was studied in rats chronically exposed to an aerosol of ammonium fluoride. The content of cytochrome P-450 and cytochrome b₅ and the activities of NADPH-cytochrome c reductase, aminophenazone N-demethylase, and aniline hydroxylase were determined. After 3 months the content of cytochrome b₅ in the F-intoxicated rats was significantly reduced by 27%, and after 6 months the activity of NADPH-cytochrome c reductase and aniline hydroxylase decreased significantly by 24 and 20%, respectively. Simultaneous oral administration of pollen extract exerted a protective effect by normalizing these parameters of the cytochrome P-450 system.

Keywords: Ammonium fluoride aerosol; Cernitin; Cytochrome P-450; Fluoride in rats; Pollen extract.

INTRODUCTION

The effects of fluoride from chronic exposure to fluorine compounds remain a leading topic in modern toxicology. Fluoride exerts its adverse action by multiple routes. Among hepatic effects already reported by us are functional disorders, alterations in lipid metabolism, and structural lesions.¹⁻⁴ The hepatic cytochrome P-450 system consists of cytochrome P-450, NADPH-cytochrome P-450 reductase, cytochrome b₅, NADH-cytochrome b₅ reductase, and phosphatidylcholine – a lipid component with an important role during electron transfer in this enzyme complex. There is considerable evidence that fluoride interferes with the function of this principal detoxication system.⁵⁻⁸

Standardized pollen extract (Cernitin) consists of a water-soluble (Cernitin T60) and lipid-soluble fraction (Cernitin GBX). The chemical composition of pollen is complex and includes vitamins, enzymes, polyphenols, minerals, phytosterols, and unsaturated fatty acids. Cernitin GBX contains from 10 to 16% of phytosterols. The commercial preparation is a mixture of both fractions.⁹

Pharmacological studies have revealed several therapeutic properties of pollen, including a hepatoprotective activity against various toxins.⁹⁻¹² Protection and stimulation of metabolism by pollen extracts were previously

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reported by us in rats chronically exposed to fluoride.\textsuperscript{13} Pollen extract alleviates biochemical changes in the liver caused by ammonium fluoride, in particular concerning lipid metabolism\textsuperscript{14} and tissue respiratory activity.\textsuperscript{13}

The aim of this work was to examine the influence of standardized pollen extract on the composition and activity of the rat hepatic cytochrome P-450 system during chronic exposure to an aerosol of ammonium fluoride.

**MATERIALS AND METHODS**

Male Wistar rats, 4 months of age, weighing 280-300 g, were randomly assigned to 8 groups with 6 to 10 rats per group and treated as shown in Table 1. The animals had free access to tap water containing 0.2 mg F/L and were fed a standard laboratory chow assayed by combustion to have 0.7 mg F/kg.

<table>
<thead>
<tr>
<th>Group</th>
<th>13 weeks Treatment</th>
<th>26 weeks Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>Unexposed controls</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>Cernitin: T60: 100 mg/kg bw/24 hr + GBX: 20 mg/kg bw/24 hr</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>NH\textsubscript{4}F: 2 mg F/m\textsuperscript{3}</td>
<td></td>
</tr>
<tr>
<td>IVA</td>
<td>NH\textsubscript{4}F + Cernitin (T60 + GBX)</td>
<td></td>
</tr>
</tbody>
</table>

Rats were exposed in a 1-m\textsuperscript{3} toxicological chamber to ammonium fluoride as an aerosol at 2 mg F/m\textsuperscript{3}, \textit{i.e.}, twice the TLV-TWA value, during 13 weeks (390 hours) or 26 weeks (780 hours). The exposure regimen was 6 hr per day, 5 days each week. The NH\textsubscript{4}F aerosol was fed into the chamber with an Ogarit JV-S inhaler. Air was exchanged 10 times per hour, and the temperature and humidity were maintained at ambient levels. The concentration of fluorine in the chamber was measured once a week using an ion-selective fluorine electrode according to Polish norm PN-83/z-04093.07. The average of 12 measurements (three-month exposure period) deviated from 2 mg F/m\textsuperscript{3} by 6.0\%, with individual values ranging from 1.6 to 2.4 mg F/m\textsuperscript{3}. The average of 24 measurements (six-month exposure period) deviated from 2 mg F/m\textsuperscript{3} by 4.8\%, with individual values ranging from 1.6 to 2.5 mg F/m\textsuperscript{3}. Operation of the aerosol system was continuously monitored by checking the volume of the NH\textsubscript{4}F solution fed into the chamber.

Standardized pollen extract (Cernitin, AB Cernelle, Vegeholm, Sweden) was administered with chow as follows: T60 (water-soluble) - 100 mg/kg body mass/24 hr; GBX (lipid-soluble) - 20 mg/kg body mass/24 hr.

Rats were weighed every two weeks. At the end of the 13- or 26-week period the animals were anesthetized with ether, and livers were removed to prepare the microsomal fraction by ultracentrifugation. The content of cyto-
chrome P-45015 and cytochrome b$_5$, and the activities of NADPH-cytochrome c reductase, aminophenazone N-demethylase, and aniline hydroxylase in the microsomal fraction were determined. Protein was quantified using Lowry’s method.

Statistical analyses were performed with the Student t test. The level of significance was taken as <0.05.

RESULTS

We noted a 10% decrease in the body mass of rats exposed for 26 weeks to ammonium fluoride as compared with controls (groups IVB vs. IB). The remaining study:control pairs, inclusive of groups exposed and simultaneously receiving Cernitin, did not differ in weight.

No significant differences were observed between unexposed animals treated with Cernitin during 13 or 26 weeks (groups IIA and IIB) and respective controls (IA and IB) as to parameters of the hepatic cytochrome P-450 system. Insignificant increases (p>0.05) in the activity of NADPH-cytochrome c reductase and aniline hydroxylase (by 11% for both) were noted (Table 2). The content of cytochrome P-450 and cytochrome b$_5$ increased insignificantly (p>0.05) after six months, by 10 and 12%, respectively (Table 3).

Table 2. Parameters of the hepatic cytochrome P-450 system (means ± SD) in rats after 13 weeks (3 months) of exposure to NH$_4$F

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P-450$^a$</td>
<td>IA 1.07±0.06</td>
</tr>
<tr>
<td>NADPH-cytochrome c red.$^b$</td>
<td>0.103±0.010</td>
</tr>
<tr>
<td>Cytochrome b$_5$$^a$</td>
<td>0.79±0.05</td>
</tr>
<tr>
<td>Amph. N-demethylase$^c$</td>
<td>4.51±0.55</td>
</tr>
<tr>
<td>Aniline hydroxylase$^d$</td>
<td>0.89±0.07</td>
</tr>
</tbody>
</table>

$^a$nmol/mg protein, $^b$µmol reduced cytochrome c/min/mg protein, $^c$nmol formaldehyde/nmol cytochrome P-450 x min, $^d$nmol p-aminophenol/nmol cytochrome P-450 x min, *p<0.05 as compared to control values (Student’s t test)

Exposure to ammonium fluoride aerosol for 13 and 26 weeks (groups IIIA and IIIB) resulted in significant alterations in the parameters of the hepatic cytochrome P-450 system as compared with respective controls (IA and IB). After 13 weeks (group IIIA), the content of cytochrome b$_5$ was reduced by 27% (p<0.05). Insignificant changes (p>0.05) included a decrease of 13% in the content of cytochrome P-450, as well as decreases of 12 and 10%,
respectively, in the activity of NADPH-cytochrome c reductase and aniline hydroxylase (Table 2). Significant decreases (p<0.05) were noted after 26 weeks (group IIIB) in the activity of NADPH-cytochrome c reductase and aniline hydroxylase by 24 and 20%, respectively (Table 3).

**Table 3.** Parameters of the hepatic cytochrome P-450 system (means ± SD) in rats after 26 weeks (6 months) of exposure to NH4F

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IB</td>
</tr>
<tr>
<td>Cytochrome P-450&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92±0.06</td>
</tr>
<tr>
<td>NADPH-cytochrome c red.&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.095±0.009</td>
</tr>
<tr>
<td>Cytochrome b&lt;sub&gt;5&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68±0.14</td>
</tr>
<tr>
<td>Amph. N-demethylase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.61±0.48</td>
</tr>
<tr>
<td>Aniline hydroxylase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.75±0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup>nmol/mg protein, <sup>b</sup>µmol reduced cytochrome c/min/mg protein, <sup>c</sup>nmol formaldehyde/nmol cytochrome P-450 x min, <sup>d</sup>nmol p-aminophenol/nmol cytochrome P-450 x min, *p<0.05 as compared to control values (Student’s t test)

Cernitin produced a normalization in the parameters of the cytochrome P-450 system in animals exposed to NH<sub>4</sub>F during 13 and 26 weeks (groups IVA and IVB) as compared with the respective controls (IVA and IB). After 13 weeks an insignificant (p>0.05) reduction (by 10%) in the activity of aminophenazone N-demethylase was observed in group IVA (Table 2). After 26 weeks the content of cytochrome P-450 insignificantly increased by 12% in group IVB (Table 3).

**DISCUSSION**

The aim of the present study was to examine whether standardized pollen extract (Cernitin T-60 and Cernitin GBX) can protect against the toxicity of ammonium fluoride.

The overall condition of animals assessed on the basis of body weight deteriorated only in the case of exposure to NH<sub>4</sub>F for 26 weeks as evidenced by a 10% weight loss in relation to the control group (groups IVB vs. IB). This finding is in accord with other reports on the toxic properties of fluorine compounds. The weight of animals in the remaining groups did not change in comparison with the controls (groups IA and IB). No significant changes in body weight were observed when animals exposed to NH<sub>4</sub>F were simultaneously given pollen extract. We are inclined therefore to attribute this result to the protective action of the pollen extract.

Significant changes were observed in the cytochrome P-450 system of ani-
mals exposed to NH₄F (groups IIIA and IIIB) in the form of reduced content of the cytochromes and activities of the constituent enzymes as compared with the respective control groups (IA and IB). The content of cytochrome b₅ was reduced by 27% after 13 weeks (Table 2). Prolongation of exposure to 26 weeks produced a decrease in the activity of NADPH-cytochrome c reductase, and aniline hydroxylase by 25 and 20%, respectively (Table 2).

Toxic effects of NH₄F seem to arise from the pro-oxidative properties of this compound. Fluorides are known to enhance peroxidation of membrane lipids. Such action may adversely affect electron transport mediated by phosphatidylcholine, the lipid component of the cytochrome P-450 system.⁵,²²,²³

Pharmacological studies have confirmed the multifarious effects of standardized pollen extracts. Therapeutic efficacy has been reported in inflammation, infections, and during convalescence.⁹,²⁴-²⁶ Pollen extracts also prevent biochemical and degenerative lesions of the liver caused by toxic factors.¹⁰-¹²

In the present study Cernitin produced a normalization in the parameters of the cytochrome P-450 system in animals exposed to NH₄F during 13 and 26 weeks (groups IVA and IVB) as compared with the respective unexposed controls (IA, IB).

The properties of Cernitin observed here appear worth considering when studying the mechanism of its beneficial effects, which include protection in chronic intoxications, as from fluoride, thereby offering promise for its prophylactic usefulness.

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