RECOVERY FROM FLUORIDE+ALUMINIUM TOXICITY IN VAS DEFERENS, SEMINAL VESICLE, AND VENTRAL PROSTATE OF MICE BY VITAMIN C

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SUMMARY: Combined administration of sodium fluoride (NaF, 10 mg/kg bw/ day)+aluminium chloride (AlCl₃, 200 mg/kg bw/day) to adult male mice for 30 days caused histological changes in the vas deferens. A decrease in protein in the vas deferens, seminal vesicles, and prostate occurred. Inhibition of phosphorylase in vas deferens together with accumulation of glycogen altered its carbohydrate metabolism. Seminal vesicle fructose and ventral prostate acid phosphatase (ACPase) also decreased causing changes in their secretions necessary for sperm function. Withdrawal of treatment for 30 days led to partial recovery of these parameters, whereas treatment with NaF+AlCl₃+vitamin C brought about complete recovery.

Keywords: Acid phosphatase; Aluminium and accessory sex glands; Fluoride and accessory sex glands; Fructose; Glycogen metabolism; Mice ventral prostate; Seminal vesicle; Vas deferens.

INTRODUCTION

The effects of aluminium+fluoride toxicity in testis and epididymis of mice after 30 days have been reported earlier.1-6 Withdrawal of treatment caused some recovery, but treatment with vitamin C together with the toxicants facilitated recovery in the respective organs. The effects of these toxicants on the vas deferens, which has absorptive, synthesizing, and secretory ability for maintenance of sperm in a viable state,7 as well as their effects on the seminal vesicles and prostate have not been adequately investigated. Hence, the present study was undertaken.

MATERIALS AND METHODS

Healthy, adult, pathogen–free, colony bred male albino mice (Mus musculus) of Swiss strain were procured and treated as described earlier5 and outlined in the following protocol:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and dose (10–12 animals in each group)</th>
<th>Duration (days)</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control+distilled water (DW)</td>
<td>-</td>
<td>a</td>
</tr>
<tr>
<td>II</td>
<td>NaF–treated (10 mg/kg bw)+AlCl₃–treated (200 mg/kg bw)</td>
<td>30</td>
<td>31st</td>
</tr>
<tr>
<td>III</td>
<td>Same as in Group II then withdrawal for an additional 30 days</td>
<td>30+30</td>
<td>61st</td>
</tr>
<tr>
<td>IV</td>
<td>Same as in Group II+Vitamin C (15 mg/animal/day) for 30 days</td>
<td>30+30</td>
<td>61st</td>
</tr>
</tbody>
</table>

aSacrificed along with treated mice.

Fluoride 2005;38(2)
After the respective treatments, the animals were sacrificed by cervical dislocation. The vas deferens, seminal vesicles, and ventral prostate were excised, blotted free of blood, and utilized for the study. Histology of vas deferens was carried out by haematoxyline and eosin (HE) staining. The levels of protein in all three organs, and glycogen and phosphorylase in vas deferens, fructose in the seminal vesicles, and acid phosphatase in the ventral prostate were determined using the methods cited.

Statistical analysis: For all biochemical parameters a minimum of 5 or 6 replicates were assayed, and the data were statistically analysed by Student’s t test.

RESULTS

Vas deferens histology: The histology of control mice vas deferens showed the epithelial folds with pseudostratified epithelium having stereocilia. Muscle layers were also seen (Figures 1 and 2). The treatment with NaF+AlCl₃ for 30 days in mice caused epithelial cell pycnosis, clumping of stereocilia, and absence of sperm bundles in the lumen (Figures 3 and 4). Withdrawal of NaF+AlCl₃ treatment for 30 days (Group III) showed some recovery with the presence of stereocilia and sperm bundles (Figures 5 and 6). Administration of ascorbic acid along with NaF and AlCl₃ (Group IV) for 30 days revealed no toxic effects since the structure of the vas deferens then appeared normal (Figures 7 and 8).

Biochemical parameters: The levels of protein decreased significantly (p<0.001) in all organs studied. Similarly, the levels of fructose in the seminal vesicles, the activities of acid phosphatase in the ventral prostate and phosphorylase in the vas deferens also declined significantly (p<0.001) in Group II animals as compared to Group I controls (Table). However, glycogen levels were increased in the vas deferens of Group II mice as compared to Group I.

The withdrawal of treatment caused recovery in all parameters with significance levels ranging from p<0.05 to p<0.01 (Table). On the other hand, vitamin C treatment along with NaF+AlCl₃ in Group IV mice promoted a very significant recovery (p<0.001) in all organs.
Figure 3. Transverse section of vas deferens of Group II (NaF+AlCl₃) treated mouse. HE staining (X 200).

Figure 4. Magnified view of Figure 3. HE staining (X 800).

Figure 5. Transverse section of vas deferens of Group III mouse showing some recovery. HE staining (X 225).

Figure 6. Magnified view of Figure 5. HE staining (X 850).

Figure 7. Transverse section of vas deferens of Group IV (NaF+AlCl₃+vitamin C) treated mouse showing marked recovery. HE staining (X 200).

Figure 8. Magnified view of Figure 7. HE staining (X 800).
DISCUSSION

At the dose levels used in the study the protein levels in all organs (vas deferens, seminal vesicle and ventral prostate) were significantly decreased, which might account for the decline in their enzyme activities and observed structural alterations, especially in the vas deferens such as epithelial cell pyknosis and clumping of stereocilia. Similar data were reported earlier using a different protocol and dose regimens of NaF and/or AlCl₃ in mice.³,⁴

The accumulation of glycogen along with inhibition of phosphorylase evidently reflects a disturbance of carbohydrate metabolism in vas deferens leading to abnormal sperm metabolism in agreement with earlier work.³,⁴ In Group II the decrease in acid phosphatase, a marker enzyme for prostate function, suggests changes in prostate metabolism,¹³ and the decrease in fructose levels in the seminal vesicles of mice indicates alteration in testosterone levels in agreement with findings of others.²-⁴

As reported in earlier communications,³-⁶ the withdrawal of treatment helped in partial recovery in all parameters, whereas addition of vitamin C resulted in complete recovery in all organs, probably because of its antioxidant, reducing properties leading to an increase in C-AMP, which promotes growth and metabolism.³,⁴

Table. Protein (mg/100 mg fresh tissue wt) in vas deferens, seminal vesicle and ventral prostate, vas deferens glycogen (µg/mg fresh tissue wt) and activity of phosphorylase (µg phosphorus released/mg protein/15 minutes), seminal vesicle fructose (µg/mg fresh tissue wt) and acid phosphatase (ACPase) (µmoles of p-nitro phenyl liberated/mg protein) in ventral prostate of Groups I to IV mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Organ</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Vas deferens</td>
<td>9.63 ± 0.21</td>
<td>4.37 ± 0.01§</td>
<td>4.97 ± 0.27*</td>
<td>6.33 ± 0.14§</td>
</tr>
<tr>
<td>Protein</td>
<td>Seminal vesicle</td>
<td>6.13 ± 0.03</td>
<td>3.30 ± 0.80§</td>
<td>5.09 ± 0.07†</td>
<td>6.07 ± 0.04§</td>
</tr>
<tr>
<td>Protein</td>
<td>Ventral prostate</td>
<td>9.45 ± 0.11</td>
<td>5.74 ± 0.42§</td>
<td>7.08 ± 0.46*</td>
<td>8.50 ± 0.25§</td>
</tr>
<tr>
<td>Glycogen</td>
<td>Vas deferens</td>
<td>974.16 ± 13.9</td>
<td>1468.34 ± 45.3§</td>
<td>1326.92 ± 41.49†</td>
<td>1032.83 ± 16.08§</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>Vas deferens</td>
<td>8.09 ± 0.01</td>
<td>5.18 ± 0.15§</td>
<td>5.98 ± 0.33*</td>
<td>7.04 ± 0.09§</td>
</tr>
<tr>
<td>Fructose</td>
<td>Seminal vesicle</td>
<td>13.61 ± 0.08</td>
<td>9.87 ± 0.76§</td>
<td>11.99 ± 0.19†</td>
<td>12.21 ± 0.07§</td>
</tr>
<tr>
<td>ACPase</td>
<td>Ventral prostate</td>
<td>0.22 ± 0.007</td>
<td>0.13 ± 0.01†</td>
<td>0.19 ± 0.03*</td>
<td>0.21 ± 0.02§</td>
</tr>
</tbody>
</table>

*a Data are expressed as mean ± SE; * P<0.05; † P<0.02; ‡ P<0.01; § P<0.001; where no sign = not significant
Comparisons: Group I with Group II; Group II with Group III; Group II with Group IV individually.
REFERENCES


