ARSENIC AND FLUORIDE INDUCED TOXICITY IN GASTROCNEMIUS MUSCLE OF MICE AND ITS REVERSAL BY THERAPEUTIC AGENTS

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SUMMARY: Sodium fluoride (NaF, 5 mg/kg bw) and arsenic trioxide (As$_2$O$_3$, 0.05 mg/kg bw), individually or in combination, were administered orally for 30 days to Swiss strain adult female mice (Mus musculus). Alterations in the physiology of the gastrocnemius muscle occurred with a decline in total protein levels and acetylcholinesterase activity that would affect its contractile pattern. The significantly enhanced levels of glycogen with a concomitant decrease in phosphorylase activity could alter the contraction of the quick contracting white fibres, while the decrease in activities of ATPase and succinate dehydrogenase suggests disturbance in the oxidative and energy metabolisms. Withdrawal of the NaF and/or As$_2$O$_3$ treatment for 30 days produced incomplete recovery. However supplementation with ascorbic acid, calcium, and vitamin E, alone or in combination, during the withdrawal period, was beneficial for recovery of the muscle parameters. These findings are important in the field of muscle physiology and kinesiology.

Key words: Arsenic and muscle; Calcium phosphate; Fluoride and muscle; Gastrocnemius muscle; Mice; Toxicity reversal; Vitamin C; Vitamin E.

INTRODUCTION

It is now well established that ingestion of fluoride affects not only teeth and bones but also many other organs in the body. Structural and biochemical changes in several soft tissues including muscle have been reported in male and female rats and mice from different doses of fluoride. Combined arsenic/fluoride poisoning also occurs, especially in India, China, and Bangladesh, where both As and F are abundant in certain drinking water sources.

Studies on the individual effects of arsenic and fluoride toxicity indicate that both cause injury to several tissues. However, the effects of the combined treatment of fluoride and arsenic in muscle tissue have not yet been reported. Therefore, it was considered worthwhile to study this aspect on the gastrocnemius muscle of female mice and the possible reversal of the induced effects by antidotes such as vitamins C and E and supplemental calcium.

MATERIALS AND METHODS

Healthy, adult female albino mice (Mus musculus) of Swiss strain, weighing between 30 and 35g were divided into thirteen groups of 10 to 12 mice each and treated according to the experimental protocol described in detail in an earlier paper. At the end of each treatment, the animals were weighed on an Ohaus SA animal weighing balance and sacrificed by cervical dislocation. The gastrocnemius muscle was dissected out carefully, blotted free of blood, and utilized for the study.

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The protein\textsuperscript{9} and glycogen\textsuperscript{10} levels, activities of succinate dehydrogenase (SDH) (E.C. 1.3.99.1),\textsuperscript{11} phosphorylase (E.C. 2.4.1.1),\textsuperscript{12} adenosine triphosphatase (ATPase) (E.C. 3.6.1.3),\textsuperscript{13} and acetylcholinesterase (E.C. 3.1.1.7)\textsuperscript{14} were determined in the gastrocnemius muscle of both control and treated animals by using the techniques cited.

For all the biochemical determinations, a minimum of 8 to 10 replicates were assayed for each parameter. The data were statistically analysed by Student’s t test and Analysis of Variance (ANOVA).

**RESULTS**

The protein and SDH levels in muscle showed a significant decline after the treatments for 30 days with NaF (SDH P<0.01), As\textsubscript{2}O\textsubscript{3}, and NaF + As\textsubscript{2}O\textsubscript{3} (P<0.001) (Groups VI to VIII) (Table 1). Withdrawal of the treatment (Group IX) resulted in an insignificant recovery in both parameters. A highly significant (P<0.001) reversal of the combined toxicity was obtained, however, after treatment with vitamin C, calcium, or vitamin E as well as their combination (Groups X-XIII) as compared to Group VIII (Table 1).

A very significant (P<0.001) accumulation of glycogen occurred in muscle by NaF, As\textsubscript{2}O\textsubscript{3}, and NaF + As\textsubscript{2}O\textsubscript{3} treatments (Groups VI-VIII). Withdrawal of treatment (Group IX) revealed a significant (P<0.01) recovery in the muscle glycogen levels as compared to Group VIII. Vitamins C or E or calcium phosphate administered alone or in combination (Groups X-XIII) resulted in highly significant (P<0.001) recovery as compared to Group VIII (Table 1).

The activities of phosphorylase, ATPase, and acetylcholinesterase declined significantly (P<0.001) with NaF, As\textsubscript{2}O\textsubscript{3}, and their combined treatments (Groups VI-VIII) as compared to the controls (Groups I-V). After withdrawal of treatment (Group IX) the activities of all three enzymes were significantly (P<0.01) recovered in comparison to Group VIII. Administration of vitamins C and E as well as calcium, alone or in combination (Groups X-XIII), resulted in an even more significant (P<0.001) recovery (Table 1).

**DISCUSSION**

Arsenic and fluoride, independently or in combination, are reported to alter protein levels in muscle tissue of treated rabbits and mice,\textsuperscript{3,7,15} probably by inhibiting its synthesis or altering its metabolism. Lack of adequate protein turnover would have an adverse effect on the receptors, structural and contractile proteins, and the activities of various enzymes in the muscle.

Arsenic and fluoride individually alter carbohydrate metabolism.\textsuperscript{16} A significant accumulation of glycogen in muscle together with a significant decline in phosphorylase activity in the present study after fluoride and arsenic treatments is in agreement with the above view.\textsuperscript{16} Glycogen is considered one of the main fuels for muscle contraction. Hence its accumulation or decreased utilization under treatment would affect the normal functioning of muscle fibres, especially the glycogen-utilizing, quick-acting, white fibres. This increase in glycogen could be due to changes in the metabolism and transport of glucose leading to hyperglycaemia in rats by fluoride administration.\textsuperscript{17}


Table 1. Protein (mg/100 mg fresh tissue wt), glycogen concentration (µg/100 mg fresh tissue wt), activities of SDH (µg formazan formed/mg protein/15 minutes), phosphorylase (µg phosphorus released/mg protein/15 minutes), ATPase (µmoles of inorganic phosphorus released/mg protein/30 minutes), and acetylcholinesterase (AChE) (AChE/mg protein) in gastrocnemius muscle of mice in Groups I-XIIIa

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Protein</th>
<th>Glycogen</th>
<th>SDH</th>
<th>Phosphorylase</th>
<th>ATPase</th>
<th>AChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control + distilled water</td>
<td>26.21 ±</td>
<td>806.53 ±</td>
<td>18.29 ±</td>
<td>10.03 ±</td>
<td>3.15 ±</td>
<td>6.31 ±</td>
</tr>
<tr>
<td>II</td>
<td>Control + olive oil</td>
<td>26.46 ±</td>
<td>804.98 ±</td>
<td>18.89 ±</td>
<td>9.92 ±</td>
<td>3.40 ±</td>
<td>6.26 ±</td>
</tr>
<tr>
<td>III</td>
<td>Control + ascorbic acid (AA)</td>
<td>26.16 ±</td>
<td>807.74 ±</td>
<td>19.27 ±</td>
<td>9.75 ±</td>
<td>3.36 ±</td>
<td>6.26 ±</td>
</tr>
<tr>
<td>IV</td>
<td>Control + calcium phosphate (Ca)</td>
<td>26.63 ±</td>
<td>807.89 ±</td>
<td>19.02 ±</td>
<td>10.18 ±</td>
<td>3.41 ±</td>
<td>6.36 ±</td>
</tr>
<tr>
<td>V</td>
<td>Control + vitamin E (Vit. E)</td>
<td>26.97 ±</td>
<td>803.75 ±</td>
<td>19.23 ±</td>
<td>9.75 ±</td>
<td>3.36 ±</td>
<td>6.26 ±</td>
</tr>
<tr>
<td>VI</td>
<td>NaF</td>
<td>17.06 ±</td>
<td>1266.67 ±</td>
<td>15.90 ±</td>
<td>7.11 ±</td>
<td>1.71 ±</td>
<td>4.20 ±</td>
</tr>
<tr>
<td>VII</td>
<td>As₂O₃</td>
<td>15.70 ±</td>
<td>1553.07 ±</td>
<td>14.25 ±</td>
<td>4.38 ±</td>
<td>1.38 ±</td>
<td>2.46 ±</td>
</tr>
<tr>
<td>VIII</td>
<td>NaF + As₂O₃</td>
<td>14.39 ±</td>
<td>1803.36 ±</td>
<td>11.27 ±</td>
<td>3.06 ±</td>
<td>1.27 ±</td>
<td>2.36 ±</td>
</tr>
<tr>
<td>IX</td>
<td>Withdrawal of Group VIII treatment</td>
<td>16.42 ±</td>
<td>1582.07 ±</td>
<td>13.48 ±</td>
<td>5.02 ±</td>
<td>1.36 ±</td>
<td>2.98 ±</td>
</tr>
<tr>
<td>X</td>
<td>Withdrawal of Group VIII treatment + AA</td>
<td>23.77 ±</td>
<td>1095.03 ±</td>
<td>16.94 ±</td>
<td>7.36 ±</td>
<td>2.15 ±</td>
<td>4.69 ±</td>
</tr>
<tr>
<td>XI</td>
<td>Withdrawal of Group VIII treatment + Ca</td>
<td>22.53 ±</td>
<td>1074.54 ±</td>
<td>14.22 ±</td>
<td>8.64 ±</td>
<td>1.66 ±</td>
<td>5.13 ±</td>
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<tr>
<td>XII</td>
<td>Withdrawal of Group VIII treatment + Vit. E</td>
<td>22.76 ±</td>
<td>1078.76 ±</td>
<td>15.01 ±</td>
<td>9.09 ±</td>
<td>1.75 ±</td>
<td>5.42 ±</td>
</tr>
<tr>
<td>XIII</td>
<td>Withdrawal of Group VIII treatment + AA, Ca &amp; Vit. E</td>
<td>26.73 ±</td>
<td>814.88 ±</td>
<td>17.92 ±</td>
<td>10.02 ±</td>
<td>3.19 ±</td>
<td>6.38 ±</td>
</tr>
</tbody>
</table>

aData are expressed as mean ± S.E.  * P<0.05; † P<0.02; ‡ P<0.01; § P<0.001; where no sign = not significant.

Comparisons between: Group I and Groups VI or VII or VIII individually; Group VIII and Groups IX or X or XI or XII or XIII individually.
Succinate dehydrogenase (SDH), a mitochondrial enzyme, catalyses the oxidation of succinate to fumarate in the Krebs cycle. Its decrease after treatment implies a block in the cycle with a probable accumulation of succinate. The reduction in activity of SDH in gastrocnemius muscle of fluoride-treated mice is in agreement with the data of others, and the results have been correlated with alterations in the structure of mitochondria and skeletal muscle fibres.

The decrease in activities of adenosine triphosphatase (ATPase) after fluoride treatment in the present study, and as reported by others, suggests that the energy metabolism is disturbed probably due to structural and functional changes in muscle. Likewise, the reduced acetylcholinesterase (AChE) activity in muscle after treatment indicates an effect on transmission of nerve impulses at the neuromuscular junctions.

In the present study, the combined treatment with NaF + As₂O₃ caused more toxicity than the individual treatments in all muscle parameters studied. An and co-workers have also reported synergism between arsenic and fluoride. Increase in serum creatinine in fluorotic humans and changes in connective tissue in rats might also be responsible for the structural and physiological manifestations in the muscle of fluorotic mice.

As noted in the results, upon withdrawal of the combined treatment, some recovery occurred in muscle, but a significant recovery in gastrocnemius muscle

Table 1a. ANOVA of various parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F-Cal</th>
<th>F-Tab</th>
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<tbody>
<tr>
<td>Protein</td>
<td>Between Groups</td>
<td>2770.8163</td>
<td>12</td>
<td>230.9014</td>
<td>86.6736</td>
<td>1.8358</td>
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<td></td>
<td>Within Groups</td>
<td>311.6920</td>
<td>117</td>
<td>2.6640</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>Between Groups</td>
<td>14764873.31</td>
<td>12</td>
<td>1230406.190</td>
<td>792.150</td>
<td>1.8358</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>181730.051</td>
<td>117</td>
<td>1553.2483</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDH</td>
<td>Between Groups</td>
<td>822.6903</td>
<td>12</td>
<td>68.5575</td>
<td>35.9964</td>
<td>1.8358</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>222.8344</td>
<td>117</td>
<td>1.9046</td>
<td></td>
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</tr>
<tr>
<td>Phosphorylase</td>
<td>Between Groups</td>
<td>714.2425</td>
<td>12</td>
<td>59.5202</td>
<td>82.8145</td>
<td>1.8358</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>84.0899</td>
<td>117</td>
<td>0.7187</td>
<td></td>
<td></td>
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<tr>
<td>ATPase</td>
<td>Between Groups</td>
<td>99.8671</td>
<td>12</td>
<td>8.3223</td>
<td>77.6420</td>
<td>1.8358</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>12.5410</td>
<td>117</td>
<td>0.1072</td>
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<tr>
<td>AChE</td>
<td>Between Groups</td>
<td>280.5569</td>
<td>12</td>
<td>23.3797</td>
<td>116.1786</td>
<td>1.8358</td>
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<td></td>
<td>Within Groups</td>
<td>23.5450</td>
<td>117</td>
<td>0.2012</td>
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<td></td>
</tr>
</tbody>
</table>

SS=Sum of squares; df=degree of freedom; MSS=Mean sum of squares; F-calc=Fisher calculated; F-tab=Fisher tabulated.
was observed by the administration of vitamins C or E or calcium phosphate either alone or in combination.

Ascorbic acid (vitamin C) is an antioxidant with detoxification properties.\(^\text{25}\) It activates adenyl cyclase but inhibits phosphodiesterase (PDE), similar to the action of calcium, which will result in an increase in c-AMP levels and cause activation of several enzymes.\(^\text{26}\) Supplementation with calcium also brought about significant recovery in numerous enzyme activities in fluoride or arsenic intoxicated mice, rats, rabbits, and guinea pigs.\(^\text{1-3,6,7}\) Calcium phosphate ingestion might have helped in overcoming the hypocalcemia induced by fluoride/arsenic treatment,\(^\text{27,28}\) and it may also have acted synergistically with vitamin C.\(^\text{5,7,29}\)

Vitamin E (\(\alpha\)-tocopherol), a potent antioxidant, exerts its protective effect primarily through destruction of cell damaging free radical oxygen species. In the present study, vitamin E also helped to reverse NaF + As\(_2\)O\(_3\) induced toxicity in gastrocnemius muscle. Together with vitamin C, vitamin E provides an additional antioxidant effect, since vitamin E interacts non-enzymatically with ascorbic acid which enhances its radical scavenging effects.\(^\text{30}\) Hence it is clear that vitamin C and vitamin E as well as calcium have an additive or synergistic effect. Thus the interaction of all three antidotes resulted in the reversal of the NaF and As\(_2\)O\(_3\) induced toxicity in gastrocnemius muscle of female mice. This is an important finding in muscle physiology and has potentially valuable implications in the field of kinesiology.

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248 Chinoy, Nair, Jhala