THE EFFECTS OF FLUORIDE, ALONE AND IN COMBINATION WITH SELENIUM, ON THE MORPHOLOGY AND HISTOCHEMISTRY OF SKELETAL MUSCLE

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SUMMARY: The objective was to study the effect of fluoride on skeletal muscle and the protection afforded by selenium. Skeletal muscle specimens from a four month old foetus were prepared for ultrastructural study after the addition of sodium fluoride, or sodium fluoride and sodium selenite, and incubation for two hours in vitro. Further specimens for ultrastructural and histochemical study were also obtained from rats who had been given, for eight weeks in vivo, drinking water containing 221 mg/L of sodium fluoride (100 ppm of fluoride), or 221 mg/L of sodium fluoride and 16.7 mg/L of sodium selenite. The histochemical results showed a decrease in the activities of the enzymes succinate dehydrogenase, cytochrome oxidase, and Mg2+-adenosine triphosphatase when fluoride had been given. The ultrastructural studies showed that the skeletal muscle cells were damaged directly by fluoride with mitochondria and myofibrils being affected. Selenium was found to protect skeletal muscle from the effects of fluoride. It was concluded that skeletal muscle cells may be damaged by fluoride with necrosis following a disruption of energy metabolism in the mitochondria with interference to the stability of the mitochondrial membrane. Selenium was found to have a protective effect which may be due to improving mitochondrial membrane stability.

Key words: Enzymes; Fluoride; Histochemistry; Selenium; Skeletal muscle; Ultrastructure.

Introduction

Skeletal muscle is sensitive to fluoride-induced muscle cell damage in chronic fluoride toxicity or fluorosis resulting in functional changes with muscle ache and weakness, and alterations to the levels of succinate dehydrogenase (SDH).1

The effect of selenium on fluorosis has been studied with no effect being found in one study2 but in another selenium alleviated lipid peroxidation by fluoride and increased fluoride excretion.3

In the present study, using in vitro and in vivo techniques, the morphological and mitochondrial enzyme changes induced by fluoride were studied together with the protective effects of selenium on fluorosis.

Materials and Methods

In an in vitro study, skeletal muscle obtained from a four month old foetus was cut into small pieces and placed in incubation bottles containing 5 mL of PRMI 1640 medium. After the addition of 50 μL of 6 mg/mL sodium fluoride, 12 mg/mL sodium fluoride, or 12 mg/mL sodium fluoride and 0.22 μg/mL sodium selenite,
the specimens were incubated for 2 h at 37°C and then placed in 3% glutaraldehyde. Control specimens were incubated in the PRMI 1640 medium or in 3% glutaraldehyde. The specimens were prepared for examination with a H-600A electron microscope.

In an in vivo study 21 male Wistar rats, weighing 180-200 g, obtained from the animal breeding facility of Harbin Medical University, were divided into three groups of 7 animals. The experimental groups were given drinking water containing 221 mg/L of sodium fluoride (100 ppm of F), or 221 mg/L of sodium fluoride and 16.7 mg/L of sodium selenite. The fluoride level of the drinking water for the control group was 0.38 mg/L (0.38 ppm of F). After 8 weeks the animals were sacrificed and the skeletal muscles placed in 3% glutaraldehyde prior to preparation for electron microscopy or immediately frozen in liquid nitrogen for histochemical study by slicing into sections 4 μm thick, incubating and staining. Six slices were tested for each enzyme for each rat. SDH was determined by Pearson's method, cytochrome oxidase (CCO) by Seligman's method, and Mg2+-adenosine triphosphatase (Mg2+-ATPase) by the method of Wachstein and Meisel. The results of the enzymatic histochemistry were analysed by the Ridit test with a higher value for the mean R indicating a higher level of enzyme activity.

Results and Discussion

In the control skeletal muscle specimens prepared in vitro, the skeletal muscle cells were slim and elongated with the myofibrils arranged orderly and showing cross striations of dark and light bands. The mitochondrial cristae were discernible (Figure 1). In the specimens treated in vitro with fluoride, most of the cells were damaged with the arrangement of the myofibrils being disorderly and some appearing shrunken, thin and necrotic. The mitochondrial cristae showed disintegration and the mitochondrial matrix was of low density (Figures 2 and 3). These changes were more serious and extensive in the specimens treated with the higher dose of sodium fluoride than in those treated with the lower dose. In the specimens receiving fluoride and selenium, the changes were less prominent than in those treated with fluoride alone (Figure 4).

For the group treated in vivo, the ultrastructure of the skeletal muscle was normal in the control group (Figure 5) but abnormal in the group treated with fluoride with localized twisting and necrosis present, together with disintegration and swelling of mitochondrial cristae (Figure 6,7). In the group treated with both fluoride and selenium, no damage to myofibrils was present but some mitochondria were swollen and hollow (Figure 8).

The Ridit test of the histochemical results showed that, when compared to the control values, sodium fluoride decreased the activities of the three enzymes studied (see Table). When selenium was present with the fluoride in the drinking water, the values for SDH and CCO were significantly higher than the values for fluoride without selenium. The value for Mg2+-ATPase increased to a non-significant extent.
Figures 1-4. Ultrastructural changes in skeletal muscle of foetus *in vitro*: 1 The control (x5000); 2 and 3 Fluoride group (x6000 and x17000); 4 Fluoride + selenium group (x6000).

Figures 5-8. Ultrastructural changes in skeletal muscle of rat *in vivo*: 5 The control (x12000); 6 and 7 Fluoride group (x4000 and x8000); 8 Fluoride + selenium group (x8000).
TABLE. The activities of SDH, CCO and Mg\(^{2+}\)-ATPase of skeletal muscle of rats treated *in vivo* with sodium fluoride and sodium selenite

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SDH 95% confidence interval</th>
<th>CCO 95% confidence interval</th>
<th>Mg(^{2+})-ATPase 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>Ridit test</td>
<td>R</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.45*</td>
<td>0.35-0.56</td>
<td>0.40*</td>
</tr>
<tr>
<td>Fluoride and Selenite</td>
<td>0.70</td>
<td>0.59-0.80</td>
<td>0.64</td>
</tr>
<tr>
<td>Control</td>
<td>0.70</td>
<td>0.60-0.81</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* compared to the control p<0.05

Conclusion

The results of the *in vitro* and *in vivo* studies indicate that mitochondria and myofibrils in skeletal muscle cells can be damaged directly by fluoride. The primary function of mitochondria is to provide energy for cells by catalyzing, with a series of enzymes, the oxidation of nutrient substances. The activity levels of the marker enzymes, SDH, CCO and Mg\(^{2+}\)-ATPase, reflect the state of oxidation and energy metabolism. In conditions with a high level of fluoride present, the stability of the mitochondrial membrane was destroyed and the activities of the marker mitochondrial enzymes decreased. It is considered that the impairment of energy metabolism may lead to the skeletal muscle necrosis which may occur with fluoride. The protective effect found with selenium may be due to an improvement in the stability of the mitochondrial membrane.

References