EVIDENCE OF MUSCLE FIBER DEGENERATION IN RABBITS TREATED WITH SODIUM FLUORIDE

by

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SUMMARY: The levels of phosphocreatine kinase (CPK) in the sera of rabbits treated with sodium fluoride were biochemically determined. The results indicate an enhanced level of serum CPK. Such an increase of CPK in the serum constitutes an index of degeneration of muscle fibers and of the highly permeable plasma membrane. The findings from the current investigation indicate that degeneration of muscle fibers results from fluoride toxicity.

The phosphocreatine content in skeletal muscles is much greater than in all other tissues in the body (1). Phosphocreatine is concentrated in mitochondria where the enzyme creatine phosphokinase (CPK) is localized (2). This enzyme is responsible for accelerating the reversible transfer of the phosphate radicle between adenosine-di-phosphate and phosphocreatine (3). Creatine phosphokinase (CPK) therefore plays an important role in phosphorylating the high energy substances in muscles.

It is known that degeneration of muscle fibers and defects of plasma membranes raise the CPK level in serum (4). The CPK level in the serum is considered an index for assessing the healthy state of the muscle fiber as well as that of the muscle membrane. Determination of the CPK concentration in the serum is, therefore, one of the methods to assess the functional status of the muscle fibers and of the plasma membranes.

In the current investigation, sodium fluoride has been administered to rabbits and serum CPK has been biochemically determined at varying time intervals.

Materials and Methods

Healthy, adult male rabbits weighing 1.1 to 1.4 kg were used.

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Muscle Degeneration

The animals were segregated into 2 groups - Group A and Group B. The rabbits of Group A comprising six animals were given 50 mg/kg body weight of sodium fluoride dissolved in 1 ml of water through the intragastric route with the aid of a fine polythene cannula 1 mm in diameter and 15 cm in length. The administration of fluoride was carried out daily up to a maximum period of 30 days. Group B comprising 8 rabbits served as controls. Both groups of animals were kept under identical laboratory conditions.

The animals of both groups were bled on day 10, 20 and 30 by puncture of the marginal vein of the right pinna. The serum collected was utilized for the estimation of creatine phosphokininase activity which was measured by the method adapted by Hughes (5) and later modified by Pearce, Pennington and Walton (6). This method is based on the quantitation of creatine formed by the interaction of creatine phosphate (CP) and adenosine-di-phosphate (ADP) as a result of creatine phosphokinase activity (CPK).

Procedure: The following were pipetted into 5 ml centrifuge tubes:

1. Serum ............................... 0.1 ml
2. Tris buffer (0.1 M; pH 7.35)........... 0.2 ml
3. Creatine phosphate (12mM)........... 0.3 ml
4. Cystein hydrochloride (0.15 M; pH 7) 0.05 ml
5. De-ionized water ...................... 0.05 ml

The test tubes were grouped into sample (I) and blank (B) and were equilibrated for 3 minutes at 37°C in a water bath. After equilibrating for 3 minutes, a solution of 0.2 ml (10 mM) was added to the sample tubes; to the tubes marked B, 0.2 ml of de-ionized water was added. These tubes were then incubated for 30 minutes. The reaction was stopped by the addition of 0.2 mg of 5% zinc sulphate and 0.2 mg of 0.3 N barium hydroxide.

The tubes containing the reaction mixture were shaken well by means of a Vortex Genie, allowed to stand for 10 minutes and then centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was decanted and 0.7 ml of it was treated with 0.3 ml of p-chloromercuribenzoic acid (25 mM), 2.5 ml of distilled water, 1 ml of x-naphthol and 0.5 ml of 0.04% diacetyl. The tubes were then allowed to stand for 5 minutes and the optical density was read at 520 mμ in a Zeiss PMQ spectrophotometer with the use of quartz cells of 1 cm path length. The results are expressed as μ moles of creatine formed/hour/liter of at 37°C (International Unit).

Results and Comments

The serum CPK values of normal rabbits, and those treated
with NaF, on day 10, 20 and 30 are reported in Table I. The results indicate a considerable rise in the level of CPK. The serum CPK of the rabbits treated with NaF were enhanced by 78%, 185%, and 276% on day 10, 20, and 30 respectively.

**TABLE 1**

<table>
<thead>
<tr>
<th>Duration of exposure to sodium fluoride</th>
<th>Amount of sodium fluoride administered (average)</th>
<th>Creatine phosphokinase level Mean + S.D.</th>
<th>Rise in CPK level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Nil</td>
<td>153.578 ± 5.10 (8)</td>
<td>78</td>
</tr>
<tr>
<td>10 days</td>
<td>0.555 gm</td>
<td>247.267 ± 7.70 (6)</td>
<td>185</td>
</tr>
<tr>
<td>20 days</td>
<td>1.078 &quot;</td>
<td>438.497 ± 7.30 (6)</td>
<td>276</td>
</tr>
<tr>
<td>30 days</td>
<td>1.390 &quot;</td>
<td>578.735 ± 11.46 (6)</td>
<td></td>
</tr>
</tbody>
</table>

The numbers given in parenthesis indicate the number of experiments carried out.

S.D. = Standard deviation.
The results are expressed in μ moles of creatine formed/hour/litre of serum at 37°C.

Hitherto, no evidence has appeared in the literature that the skeletal muscle is directly affected in fluorosis. The involvement of the muscle has been considered to be a secondary effect resulting from the compression of the spinal cord and of nerve roots because of narrowing of the vertebral canal and the intervertebral foramina (7). The current study, however, indicates that as early as 10 days after administration of sodium fluoride, the creatine phosphokinase activity increases in the serum by 78%. The rise in CPK has been found to be a gradual process and the level shot up to 276% by day 30. This progressive increase in CPK in serum proves that the direct involvement of muscle is due to fluoride toxicity. This conclusion is supported by the fact that our light microscopic studies on the mammalian diaphragm have failed to disclose any structural changes suggestive of neuronal degeneration in muscle (8).

It is noteworthy that the serum level of CPK is very high during the onset and early phases of muscular dystrophy (9, 10) and that, during this phase, the population of muscle fibers undergoes the maximum structural damage. During the late stages of dystrophy, when the degeneration of muscle fibers is almost complete, and when the muscle is fully infiltrated with fat and connective tissue, the serum CPK levels are known to be well below the normal range. This evidence supports the view that the rise in serum CPK level could be due to degeneration of muscle fibers.
muscle fibers.

It may be assumed that the increased levels of CPK in serum could be due to atrophy of muscle fibers of the neuronal type. But the rise in the level of serum CPK is recorded only during the late stages of neurogenic atrophy, not during the early phase of the disease (10). The changes in serum CPK recorded in the NaF-treated animals is not likely to be related to neuronal involvement because a 30 day period is too short a time to produce such a high % rise. Moreover, as indicated above, our light microscopic studies have not revealed any evidence of neurogenic atrophy of muscle tissue.

Our recent histochemical and ultrastructural studies on rabbit diaphragm exposed to toxic doses of sodium fluoride constitute additional evidence that degeneration of muscle fibers and destruction of mitochondria occur in fluoride toxicity even at very early stages (8). The current experimental study, therefore, indicates that degeneration of muscle fibers in 'Fluorosis' is most likely due to the direct action of fluoride on the skeletal muscle rather than to secondary action which is the result of compression upon the spinal cord.

Bibliography


FLUORIDE


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