HISTOPATHOLOGY OF MYOCARDIAL DAMAGE IN EXPERIMENTAL FLUOROSIS IN RABBITS

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Summary: Young albino rabbits were administered 5, 10, 20, and 50 mg of sodium fluoride/kg body weight/day subcutaneously for 3.5 months. The control animals were given 1 mL of double distilled water/kg body weight/day. In the fluoridated rabbits, the myocardium showed cloudy swellings, sarcoplasmic vacuolization, and small hemorrhages followed by fibrous necrosis. The degenerative changes were most pronounced in animals treated with 50 mg of sodium fluoride/kg body weight/day. The myocardium exhibited fibrous necrosis, dissolution of nuclei, fibrillolysis, extensive vacuole formation and interstitial cells in the connective tissue. The degree of myocardial damage seemed to be directly proportional to the dosage of fluoride administered. In the control animals, the myocardium showed normal structure without any of the changes mentioned above.

Keywords: Acute fluoride intoxication, Albino rabbits, Heart histopathology, Myocardial damage, Sodium fluoride.

INTRODUCTION

Although acute toxic effects of fluoride on the heart are fairly well known, information about the exact nature of these effects as well as chronic effects is still very limited. In experimental animals given massive doses of fluoride, cardiac irregularities and low blood pressure have been reported.[1,2] Changes in the electrocardiogram and heart enlargement in children were linked to fluoride in the drinking water,[3] but there is less certainty about the nature of these effects.

Previously, we found that experimental fluorosis in rabbits produced pathological lesions in the trachea,[4] pulmonary damage,[5] hypertrophy and hyperplasia in skeletal muscle,[6] functional sterility,[7,8] and structural alterations in the lens.[9] The present study was undertaken to examine myocardial damage in albino rabbits resulting from acute and chronic fluoride intoxication.

MATERIALS AND METHODS

A total of 60 albino rabbits of both sexes weighing 400-650 g were used in these experiments. They had free access to food (rabbit pellet chow) and normal low-fluoride tap water *ad libitum.*

Experimental design: The animals were divided into five groups of 12 animals each. Four groups were administered 1-mL subcutaneous injections of sodium fluoride (NaF) in doses of 5, 10, 20, and 50 mg/kg body weight/day for 3.5 months. The control rabbits were administered 1 mL of double distilled water/kg body wt/day. All animals were weighed weekly. At the end

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of the experiment, they were sacrificed under ether anaesthesia, and their hearts were removed for histopathological study.

Histopathology: Small samples of cardiac muscle were fixed in alcoholic Bouin's fixative and Carnoy's fluid, washed in 70% alcohol, dehydrated in tert-butyl alcohol, cleared in amyl acetate, infiltrated, and embedded in paraffin. They were serially sectioned at 7 µm and stained with iron haematoxylin and eosin. Photomicrographs were taken with an Olympus camera attached to a trinocular Olympus microscope.

RESULTS

Histopathological examination of the heart sections showed clear differences between controls and experimental animals. In animals treated with 5 mg of NaF/kg/day, the myocardium showed cloudy swellings and sarcoplasmic vacuolisation as compared to the controls (Figure1). There were also small haemorrhages, interstitial oedema, and fibrous necrosis in the myocardium (Figure 2).

Figure 1. Longitudinal section through the heart of a rabbit showing normal structure of cardiac muscle in the control group x 400

Figure 2. Longitudinal section through cardiac muscle showing fibrous necrosis, interstitial oedema, and damage to parenchyma in a rabbit treated with 5 mg of NaF/kg body weight x 400

Cloudy swellings of myocardium appeared in all the rabbits given 10 mg of NaF/kg/day. The cardiac muscle showed fibrous necrosis and fibrillolysis (Figure 3). There was clear evidence of interstitial oedema and infiltration of lymphocytes, histiocytes, and granulocytes. In the interstitial spaces, an edematous fluid was present. At some places, there was extensive vacuole formation (Figure 4).

In animals treated with 20 mg of NaF/kg/day, cloudy swellings of fibres, fibrous necrosis, fibrillolysis, sarcoplasmic vacuolisation, and dissolution of nuclei (Figure 5) occurred in the myocardium. Interstitial oedema and round-cell infiltration into the connective tissue was more advanced. The myocardial degenerative changes were more pronounced as compared to animals treated with 5 and 10 mg of NaF/kg/day.

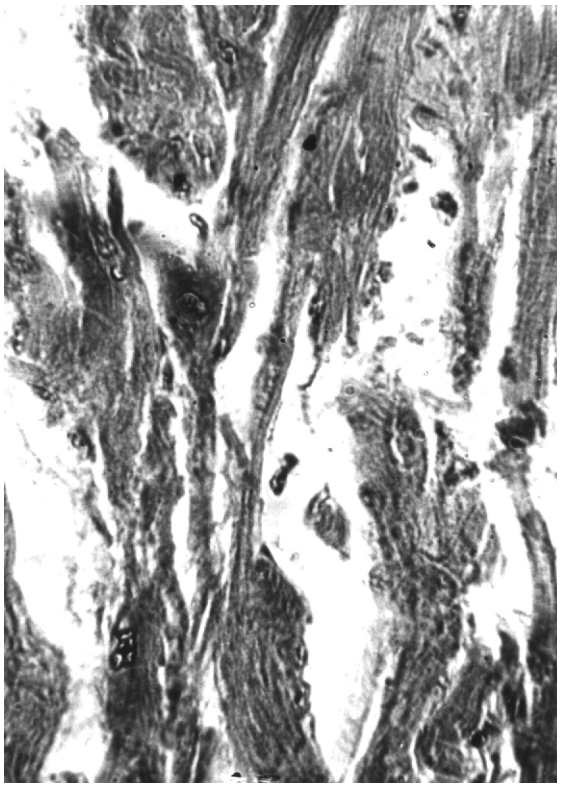

Figure 3. Longitudinal section showing oedema of muscle fibres and fibrillolysis in cardiac muscle of a rabbit treated with 10 mg of NaF/kg x 400

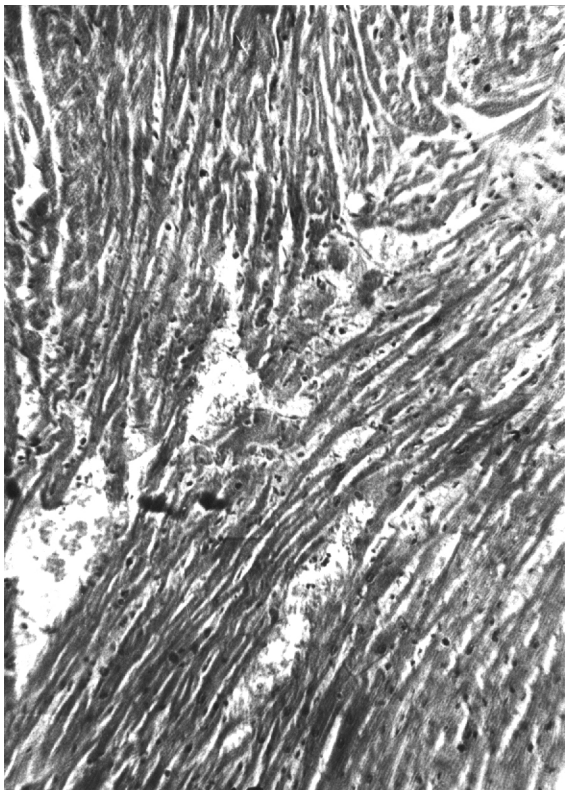

Figure 4. Photomicrograph showing formation of vacuoles and interstitial oedema in cardiac muscle of a rabbit treated with 10 mg of NaF/kg x 400

Figure 5. Photomicrograph showing extensive fibrous necrosis, sarcoplasmic vacuolisation and dissolution of nuclei in cardiac muscle of a rabbit treated with 20 mg of NaF/kg x 400

Figure 6. Photomicrograph showing fibrous oedema, clumped fibres, fibrillolysis and interstitial oedema in myocardium of a rabbit treated with 50 mg of NaF/kg x 400

The degenerative changes in the myocardium were most marked in the rabbits treated with 50 mg of NaF/kg/day. The pathological changes included fibrous necrosis, dissolution of nuclei, fibrillolysis, formation of vacuoles, and interstitial oedema (Figures 6, and 7). The degree of myocardial damage appeared to be directly proportional to the dosage of fluoride administered. In the control animals, the myocardium showed normal structure (Figure 1) and none of the changes mentioned above were recorded.

Figure 7. Acute fibrous necrosis, dissolution of nuclei and formation of vacuoles in cardiac muscle of a rabbit treated with 50 mg of NaF/kg for 70 days x 400

DISCUSSION

In the present investigation, pathological changes, *viz.* extensive interstitial oedema, fibrous necrosis, and cloudy swellings were significant in the myocardium of all fluoridated rabbits of each group. In the highest dosage

group (50 mg of NaF/kg/day), the disintegration of myocardial fibres, round cell infiltration, and acute hemorrhages were more intensely marked.

Okushi[10] reported cloudy swellings, infiltration with round cells, thickening of adventitia, diffuse hemorrhages, and vacuolar and colloid degeneration of myocardium in rabbits fed 10-100 mg of NaF for 132 days. The changes were more prominent in the papillary muscles and on the inside wall of the myocardium than at the exterior cardiac wall. In rats treated with 0.71 to 31.03 mg/kg of fluoride for one month, Takamori[1] reported the presence of cloudy swellings, vacuolar degeneration, round-cell infiltration, and hemorrhages and, in rabbits, regressive degeneration, cellular infiltration, hyperaemia, hemorrhages, and thickening of the vessel walls in the heart muscle. Almost the same changes were observed in the cardiac muscle of fluoride-intoxicated rabbits during the present investigation.

In humans, Okushi[3] found a higher incidence of myocardial damage electrocardiographically and cardiac dilatation roentgenocardiacally in inhabitants of a high-fluoride zone, where the drinking water contained 6-13 ppm fluoride. In residents of a Japanese village, where the fluoride levels ranged between 0.5 and 6.2 ppm, Takamori *et al*[11] reported myocardial damage and dilatation of the cardiac muscle and established a direct relationship between increased myocardial damage and mottled enamel by means of electrocardiograms. In acute fluorosilicate poisoning, Muller and Bock[12] observed oedema of the myocardium with diapedesis of erythrocytes and leukocytes, along with acute right dilatation of the heart, in addition to a general venous hyperaemia. Degenerative changes consisting mainly of fragmentation of muscle fibres in the heart of a fluorotic patient were reported by Fasske,[13] who noted the replacement of delicate sarcolemma by fibrous structure.

During our experiments, fragmentation and degeneration of cardiac muscle fibres were observed. However, the replacement of sarcolemma by fibrous tissue did not occur. Pribilla[14] noticed fibrous necrosis, dissolution of nuclei, fibrillolysis, interstitial oedema, minute hemorrhages and infiltration of histiocytes, lymphocytes, and granulocytes in the myocardium of patients with acute silicofluoride intoxification. We also saw these same types of degenerative changes in cardiac muscle.

Since there are degenerative changes in myocardial fibres in acute poisoning due to massive doses of fluoride, the effect of persistent minute doses of fluoride intake over prolonged periods cannot be ignored. Variations in the occurrence of abnormalities in the fluoridated myocardium may be explained on the basis of differences in the dose and period of administration. The present investigation suggests that fluoride interferes with myocardial metabolism, a condition observed by Iwase,[14] who demonstrated histochemically that fluoride caused degenerative changes in the myocardium of

rabbits administered 10-30 mg of NaF/kg/day orally for 15-169 days, followed by changes in the localisation of glycogen.

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