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Red Blood Cell Glucose Metabolism in Human Chronic Fluoride Toxicity

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Fluoride is a well known inhibitor of many enzyme systems in vitro (Shahed et al. 1979; Messer 1984). The most widely studied classic example of fluoride inhibition is its potent inhibition of glycolysis, specifically its action on the enzyme enolase (Warburg and Christian 1941; Shearer and Suttie 1970; Repaske and Suttie 1979; Shahed et al. 1979). Despite the plethora of in vitro studies on the effects of fluoride on the enzyme activity, there is a paucity of information concerning the in vivo metabolic lesions caused by the chronic toxic doses of fluoride in humans. The present study has been undertaken with a view to assess the changes in glucose metabolism and related enzymes in erythrocytes of humans consuming toxic doses of fluoride for prolonged periods.

MATERIALS AND METHODS

Ten male members of the age group 20 to 35 years with severe manifestations of skeletal fluorosis from Ralla Anantapuram village, Anantapur district, Andhra Pradesh, India, who have been consuming drinking water with high fluoride content (7.2 to 10.7 ppm) since birth were chosen as subjects for study. Healthy males of same age and socioeconomic status residing in neighboring villages with permissible content of fluoride in water (0.5 to 1.0 ppm) served as controls.

Blood was collected by venipuncture in EDTA Na_2 (1mg/ml) and red cells were isolated by repeated washings with phosphate buffered saline (nine parts 0.9% NaCl; one part 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 7.4). The flux of glucose through the hexose monophosphate (HMP) shunt was assessed essentially by the method of Trotta et al. (1982) and glycolytic activity was measured by measuring the lactate produced in the medium. 2.0 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 3.75 mM D-glucose with 0.5 µCi of [U-^{14}C]-D-glucose and 2.0 ml of 1% (V/V)

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red cell suspension in Krebs-Ringer phosphate buffer was incubated at 37°C for 1 hour. The $^{14}CO_2$ trapped by KOH layered on a filter paper was counted for radioactivity (Robyt and White 1987) and the lactate content in the medium was estimated spectroscopically (Beutler 1975).

Glucose-6-phosphate dehydrogenase, pyruvate kinase and lactate dehydrogenase activities were determined according to Beutler (1975) in red cell hemolysates.

Protein was determined by the method of Lowry et al.(1951) using bovine serum albumin(sigma) as standard and haemoglobin was estimated by using Drabkin's reagent (Henry, 1984).

The statistical significance of the data was evaluated by student "t" test.

RESULTS AND DISCUSSION

The metabolism of glucose through glycolytic pathway, pentose phosphate pathway have been assessed in erythrocytes of control and fluorotic patients and presented in table 1. Erythrocytes from control humans produced 8.55 ±0.44 µ moles of lactate/g protein/hour, when incubated with 3.75 mM D-glucose. However, under similar conditions the red cells from fluorotic patients produced only 2.61 ±0.20 µ moles of lactage/g protein/hour. Thus chronic intoxication with fluoride appear to result in inhibition of glycolysis in erythrocytes nearly by 70%.

Table 1. *In vivo* and *in vitro* effect of fluoride on human erythrocyte glucose metabolism.

Source of Erythrocytes	Addition	Flux through glycolysis µ moles of lactate formed/g protein/hr.	Flux through HMP shunt CPm/g protein/hr.
Control	--	8.55±0.44 (7)	2780.4±67.3 (6)
Fluorotic Patients	--	2.61±0.20[a] (7)	19456.7±2029.9[a](6)
Control	0.1 mM F	7.45±0.30[b] (5)	--
Control	1.0 mM F	6.51±0.45[b] (5)	--
Control	10.0 mM F	5.04±0.19[a] (5)	14927.4±837.1[a] (6)

Values are mean ±S.E. number of experiments given in parenthesis. a $P < 0.001$ b $P < 0.05$.

Studies were extended to confirm the earlier findings of Shearer and Suttie (1970); Shahed et al. (1979) to demonstrate the inhibition of glycolysis in normal erythrocytes by fluoride. The effect of fluoride was studied by adding variable amounts of fluoride (0.1 to 10.0 mM F) to red cell suspension from normal healthy adult males with 3.75 mM D-glucose and measuring the rate of production of lactate and the results are presented in table 1. The present study confirms the inhibition of glycolysis, in red blood cells by fluoride in vitro. A perceptible inhibition of 11.4% was observed even with 0.1 mM F and increasing the fluoride concentration to 1 mM and 10 mM resulted in 22.7% and 40.1% inhibition, respectively.

HMP shunt activity was assessed in intact erythrocytes of control and fluorotic patients by measuring the release of $^{14}CO_2$ from [U-^{14}C]-D-glucose. HMP shunt activity was increased by seven fold in the erythrocytes of fluorotic patients (Table 1). Since 10 mM F was found to inhibit glycolysis in vitro by 40% in erythrocytes from normal healthy persons (Table 1) the effect of an additional 10 mM F on pentose phosphate pathway in red cell suspension from normal healthy adult males was investigated and found to be a 5.4 fold stimulation. Thus the present study revealed a similar pattern of alterations are brought about by fluoride either in vivo or in vitro on glucose metabolism in erythrocytes.

Having examined the changes in the HMP shunt and glycolytic pathways in erythrocytes of individuals with chronic fluorosis, changes in the activities of some of the enzymes of these pathways viz., pyruvate kinase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase were investigated.

Table 2. Activities of Pyruvate kinase, Lactate dehydrogenase and Glucose-6-phosphate dehydrogenase in erythrocytes of Control and Fluorotic Patients.

Item	Control	Fluorotic patients
Pyruvate kinase (IU/gHb) (6)	18.49 ± 0.95	13.49[a] ± 0.63
Lactate dehydrogenase (IU/gHb) (10)	198.35 ± 1.11	114.90[a] ± 8.08
Glucose-6-phosphate dehydrogenase (IU/gHb) (6)	8.66 ± 0.48	10.76[a] ± 0.18

Values are mean ± S.E. of number of experiments given in Paranthesis. a $P < 0.001$.

The activities of the two glycolytic enzymes, pyruvate kinase and lactate dehydrogenase were significantly decreased in erythrocytes of fluorotic patients (Table 2). The observed decrease in the activity of pyruvate kinase was 28% and lactate dehydrogenase was 42%. The activity of the key enzyme of pentose phosphate pathway, glucose-6-phosphate dehydrogenase, was found to be increased by 24% in red cells of these patients compared to controls (Table 2).

In unstressed situation, 5 to 10% of the total glucose consumption of human red cells is channeled via HMP shunt (Gaetani et al. 1974; Beutler 1975). The flux is limited by the first enzyme of the cycle, glucose-6-phosphate dehydrogenase, which owning to inhibition by high NADPH/NADP ratio, operates at less than 1% of its capacity (Thorburn and Kuchel 1985). However, under conditions of increased NADPH utilization the NADPH/NADP ratio decreases and the inhibition of glucose-6-phosphate dehydrogenase is released. Such a situation appear to have arisen in erythrocytes of fluorotic patients due to enhanced requirement of NADPH for reduction of oxidized glutathione. This might have resulted in the observed increase in oxidation of glucose through HMP shunt and increase in glucose-6-phosphate dehydrogenase activity in the erythrocytes of fluorotic patients.

The diminished activities of pyruvate kinase and lactate dehydrogenase in the erythrocytes of fluorosed subjects suggests that fluoride can inhibit enzymes other than enolase. Prolonged *in vivo* exposure of red cells to high oxygen pressures have been shown to result in enhanced lipid peroxidation and inhibition of glyceraldehye-3-phosphate dehydrogenase (Mengel and Kann 1966). Since fluoride *in vivo* and *in vitro* has been demonstrated to cause increased lipid peroxidation in erythrocytes of humans (Saralakumari 1990) and in tissues of fluorosed animals (Antonyan 1980; Soni et al. 1983) it is to be expected that inihibition of glycolysis by fluoride may be a result of complex phenomena. The *in-vivo* inhibition of glycolysis suggests that fluoride can inhibit enzymes other than enolase. In rat erythrocytes, a Ca^{2+}-activated protease is implicated in the regulation of pyruvate kinase (Dahlqvist and EkMan 1981). An enzyme may be inhibited by fluoride either directly (Slater and Bonner 1952) or through a change in the concentration of such divalent cations as Ca^{2+}, Mg^{2+}, Mn^{2+} in the medium (Lu et al. 1961). Results concerning the effect of fluoride on the metabolism of these ions in animals are inconclusive. Leone et al. (1956) found that large dose of NaF given intravenously to dogs slightly reduced the calcium level in blood. Simpson et al. (1980)

reported severe hypocalcemia in a patient following ingestion of fatal dose of fluoride. The similarity of fluoride ion (F^-) to hydroxyl ion (OH^-) in terms of ionic radius and primary hydration number led (Nowak and Maurer 1981) to suggest that F^- serves as a possible analog of OH group involved in the gain or loss of water as part of normal reaction mechanism. Inhibition of enolase is suggested to be due to the formation of a tightly bound enzyme-metal-F-Pi complex in which the F^- interacts directly with the metal ion in the active site and indirectly with the phosphate binding site (Nowak and Maurer 1981).

Chronic fluoride ingestion leads to elevation and accumulation of fluoride in bone (Messer 1984), an erythropoietic tissue. The changes in glucose metabolism in erythrocytes of these patients may be related either to the direct effect of fluoride and/or due to structural and functional alterations of red cells during erythropoiesis by accumulated fluoride in bone.

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