Chemosphere, Vol.16, No.5, pp 1087-1094,1987
Printed in Great Britain

0045-6535/87 $3.00 + .00
Pergamon Journals Ltd.

EFFECT OF SODIUM FLUORIDE - ON ERYTHROCYTE MEMBRANE FUNCTION - WITH REFERENCE TO METAL ION TRANSPORT IN RABBITS

Suresh K. Jain and **A.K. Susheela** *
Fluoride and Fluorosis Research Laboratory
Department of Anatomy
All-India Institute of Medical Sciences
New Delhi-110 029, INDIA.

ABSTRACT

Effect of NaF on membrane function was examined with reference to metal ion transport in rabbit's erythrocytes. Activities of membranal enzymes [Na^+ + K^+ - ATPase & Mg^{++} - ATPase], plasma enzymes [Acid and Alkaline Phosphatases] and concentrations of Ca, Mg, Na and K were estimated in erythrocyte membrane, hemolysate and plasma. A 17% decrease in Na^+ + K^+ - ATPase and 37% increase in Mg^{++} - ATPase have been observed after fluoride ingestion. Plasma, Acid and Alkaline Phosphatases are reduced by 27% and 34% respectively. Fluoride in circulation and in erythrocyte membrane do not effect the concentration of Na and K, however, Ca and Mg are significantly altered in erythrocyte membrane, hemolysate and plasma. It is concluded that fluoride is affecting the normal erythrocyte membrane function.

INTRODUCTION

Under normal circumstances erythrocyte membrane is known to regulate the transfer of ions between internal and external environment of the cell. Transfer of cations is thought to be through specific membrane channels [1]. However, the mechanism of transfer of sodium and potassium ions is linked to a pump i.e., Na - K pump of membrane. Transfer of sodium and potassium through Na - K pump is regulated by an enzyme system, ATPase [2]. Similarly, the transfer of calcium and magnesium is regulated by another ATPase, which is independent of [Na^+ - K^+]-ATPase of the membrane [3].

The erythrocyte membrane is known to accumulate fluoride and undergoes certain morphological changes leading to "Echinocyte" formation during fluoride toxicity [4 & 5]. The inhibition of activities of enzymes, glucose-6-phosphate dehydrogenase and pyruvate kinase in rabbit erythrocytes [6] and stimulation of phosphofructokinase in human erythrocytes by NaF are also reported [7].

Alterations in metabolism of calcium and magnesium by fluoride in liver, kidney and bone have been reported by several investigators [8-10]. According to Suketa et al [23], a decrease in the concentration of sodium and potassium is observed in the serum of fasted rats administered a single oral dose of fluoride, due to increased excretion of these ions in urine.

However, the manner in which the intracellular fluoride in erythrocytes affect the ion mobilization is not well understood. Therefore, in the present study, effect of sodium fluoride on the mobilization of metal ions - sodium, potassium, calcium and magnesium across the erythrocyte membrane is evaluated and results reported.

MATERIALS AND METHODS

Ten female albino rabbits weighing 800-1050 gm. each were segregated randomly into two groups of five each and were fed a balanced diet obtained from Hindustan Lever [Bombay]. The animals in the first group were administered, NaF 10 mg/kg body weight, per os, daily for six months. Animals in the second group served as age and sex matched controls. At the end of 6 months, the animals were anaesthetized using ether and blood was drawn from jugular vein into beakers containing heparin [sodium salt] which acted as anticoagulant. Samples were centrifuged after stabilization at $4^{o}C$ at 200 X g for 10 minutes and plasma separated was used for further investigations. All subsequent centrifugations were also carried out at the same temperature.

Membrane preparation :

The erythrocyte membrane was prepared by a modified method of Bond & Green [11]. The cells were washed three times in 6 volumes of 0.16 M Tris HCL [pH 6.8]. After third washing the buffy coat was removed and the cells were hemolyzed in 5 volumes of ice cold 1.0 mM Tris HCL [pH 7.5]. The hemolysate was centrifuged at 2500 X g for 15 minutes. The pellet was washed several times in ice cold 10 mM Tris HCL [pH 7.5]. Washing was continued until the membrane preparation was light pink or nearly white in colour. The membrane preparation was then taken in a medium consisting 10 mM imidazole which had been adjusted to [pH 7.2] with HCL. This was stored and used for further analysis.

ATPase activity :

ATPase activity of erythrocyte membrane was assayed by measuring the inorganic phosphate liberated using a modification of the method of Fiske and Subba Row [12] as adopted by Bond and Clough [13].

The incubation was carried out in 15 ml plastic tubes. The reaction mixture contained : 1.5 mM Tris ATP, 1.7 mM Mg^{2+} and 38 mM Tris HCL [pH 7.8]. The final volume was 2 ml. Sodium and Potassium activated ATPase activity was separated with and without addition of Ouabain to the reaction mixture. At the end of the incubation, the reaction was stopped by 1.2 M perchloric acid and the supernatant was used for the estimation of the inorganic phosphate.

Acid and Alkaline Phosphatase activities :

The activities of acid and alkaline phosphatase from plasma, hemolysate and erythrocyte membrane were assayed according to the method of Linhardt and Walter [14] using p-nitrophenylphosphate as substrate.

Estimation of Sodium, Potassium, Magnesium and Calcium :

Sodium and potassium ions from plasma, hemolysate and erythrocyte membrane were estimated using a Elico Flame Photometer, Model CL-22 euipped with sodium and potassium specific filters as described by Prasad, A.S. [15].

The concentrations of calcium and magnesium ions in plasma, hemolysate and erythrocyte membrane were determined with an Atomic Absorption Spectrophotometer [Pye Unicam Model SP-900] as described by Prasad, A.S. [15] and Barker, et al [16]. The precision was checked by using internal standards. The glassware and sampling bottles were cleaned in hot nitric acid to remove all possible contaminations.

Determination of fluoride content :

Fluoride content from hemolysate, plasma and the membrane was determined by using a Radiometer PHM-84 with a fluoride specific electrode by the method of Hall et al [17].

Protein from membrane samples was estimated by the method of Lowry, et al [18]. Hemoglobin content of the hemolysate was determined by using a commercial Sigma kit No. 1052. The statistical analysis of the data was done employing Student's 't' test.

RESULTS

The results on $[Na^+ + K^+]$-ATPase and Mg^{++}-ATPase activities in erythrocyte membrane before and after fluoride ingestion are presented in Table 1. The activity of $[Na^+ + K^+]$-ATPase is reduced [17%] and there is a simultaneous increase [37%] in the activity of Mg^{++}-ATPase in erythrocyte membrane after fluoride ingestion.

Table 1 : Effect of Sodium Fluoride on the ATPase activities of Erythrocyte Membrane[a] [Data expressed - Mean ± S.D.]

	$[Na^+ + K^+]$-ATPase	Mg^{2+}-ATPase
NORMAL	0.17 ± 0.02	0.27 ± 0.02
6 MONTHS FLUORIDE TREATED	0.14 ± 0.01*	0.37 ± 0.01*

a = Each set of reading is mean of 5 experiments

* = P value < 0.05

Activity is expressed as n moles Pi released per h per mg protein

Data in Table 2 show the activities of acid and alkaline phosphatases in erythrocyte membrane, hemolysate and plasma. The activity of these two enzymes remained unaltered in erythrocyte membrane after fluoride treatment. However, in plasma and hemolysate the activity of acid phosphatase was decreased significantly. The activity of alkaline phosphatase was negligible in hemolysate and could not be recorded.

Table 2 : Effect of Sodium Fluoride on the Activity of the Enzyme Acid Phosphatase and Alkaline Phosphatase in Plasma, Hemolysate and Erythrocyte Membrane[a] [Data expressed-Mean ± S.D.]

	Acid Phosphatase		Alkaline Phosphatase	
	Normal Rabbits	Rabbits treated with NaF	Normal Rabbits	Rabbits treated with NaF
Plasma	32.6 ± 3.1	25.8 ± 2.7*	41.7 ± 2.7	27.8 ± 3.6*
Erythrocyte Membrane	4.8 ± 1.4	4.3 ± 1.0	15.0 ± 1.0	15.6 ± 1.9
Hemolysate	24.9 ± 3.9	21.1 ± 2.3*	n.d.	n.d.

a = Each set of reading is mean of 5 experiments;
* = P value < 0.05
n.d. = Activity not detectable
The enzyme activity is expressed in terms of n mole of paranitrophenol released at 37°C/hour

The concentrations of Ca, Mg and Na ions in erythrocyte membrane are shown in Fig. 1. It is evident that the Ca and Mg are reduced after fluoride treatment. While, there is no change in the concentration of Na. However, K was not detectable in erythrocyte membrane of normal and treated samples.

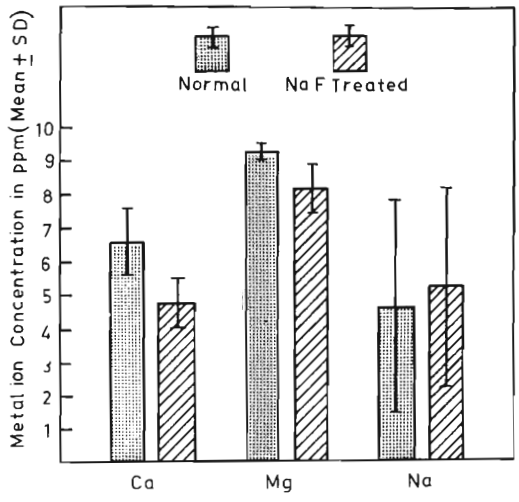

Fig. 1 :
Concentrations of Ca, Mg & Na in Erythrocyte Membrane/mg protein.

The concentration of Ca, Mg, Na and K ions in hemolysate and plasma are reported in Table 3. Results show no significant change in the concentration of Na and K in hemolysate and plasma after fluoride treatment. However, the concentration of Ca ions increased in plasma and hemolysate after six months of fluoride treatment. On the other hand, the concentration of Mg was reduced in plasma and significantly enhanced in hemolysate of fluoride treated samples.

Results presented in Table 4 indicate an increase of 33.3% of fluoride in erythrocyte membrane of rabbits administered NaF [10 mg/kg body weight] for six months.

Table 3 : Effect of Fluoride Ingestion on Metal Ion Concentration in Hemolysate and Plasma in ppm[a] [Data expressed, Mean ± S.D.]

	PLASMA				HEMOLYSATE[b]			
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
Normal Rabbits	61.6±6.7	97.3±8.9	42±5.6	65.8±9.0	6.4±1.31	4.4±0.50	77.9±14.8	32.6±5.3
Rabbits treated with	76.3±5.1*	71.7±5.9*	42.4±2.6	62.2±3.8	9.1±0.9*	7.0±1.7*	80.5±12.9	32.9±6.5

a = Each set of reading is mean of 5 experiments;
b = Hemoglobin = 5.7 gm%;
* = P value < 0.05;
S.D. = Standard deviation

DISCUSSION

The present study demonstrates that the activity of acid phosphatase in plasma and Na^{+} and K^{+} activated ATPase in the erythrocyte membrane is inhibited by fluoride. In an earlier study, we have also shown that fluoride is accumulated in erythrocyte membrane of rabbits administered sodium fluoride [10 mg/kg body weight] for 6 months [Table 4]. From the observations, it is concluded that sodium pump of erythrocyte membrane is directly inhibited by fluoride. Increased activity of Mg^{++} activated ATPase is perhaps due to reduction in endogenous calcium in membrane [Fig. 1]; as it has been suggested by Vencenzi [19]. According to Vincenzi endogenous calcium regulates the activity of Mg^{++} activated ATPase in membrane. Mg^{++} activated ATPase controls the mobilization of magnesium in erythrocytes. Therefore, the enhanced activity of this enzyme will increase the concentration of magnesium in erythrocytes as reported in the present study.

Table 4 : Fluoride Content of Rabbit Erythrocyte, Membrane, Hemolysate and Plasma [Data expressed, Mean ± S.D.]

	Fluoride in ppm [Normal]	Fluoride in ppm [After 6 months treatment]	percentage increase
Erythrocyte membrane[a]	0.03 ± 0.03	0.046 ± 0.005*	33.3
Hemolysate [b]	0.001 ± 0.004	0.08 ± 0.013*	
Plasma	0.05 ± 0.03	0.48 ± 0.08**	

a = 10 mg protein/ml membrane suspension b = ppm F^-/gm% hemoglobin of hemolysate

* = P $<$ 0.05 ** = P $<$ 0.005

Increase in concentration of calcium in hemolysate may either be due to change[s] at erythrocyte membrane leading to increased Ca^{++} permeability or decreased activity of Ca-ATPase pump which extrudes Ca^{++}. Several chemicals are known to increase the permeability of membrane for Ca^{++} under various physiological conditions [20 & 21]. However, the effect of fluoride on membrane permeability is still an enigma. On the other hand, Mg^{++} regulates the activity of Ca-ATPase in erythrocytes [22]. Therefore, it is suggested that the increased concentration of Mg in hemolysate might be inhibiting the activity of Ca-ATPase of the membrane and because of the decreased efficiency of Ca-ATPase pump, calcium is accumulated in erythrocytes.

The results on metal ion concentration in erythrocyte membrane, hemolysate and plasma suggests that fluoride alters Ca and Mg concentrations in blood but causes no significant change[s] in Na and K contents. Hence, the hemeostatic regulation of these ions is strongly maintained during fluoride toxicity. Suketa et al [23] have shown a similar regulation of Na and K in rats after a single large dose of fluoride. In conclusion, it may be stated that fluoride alters the activity of membranal enzymes affecting the transport of calcium and magnesium in erythrocytes.

ACKNOWLEDGEMENT

One of the authors [A.K. Susheela] wishes to acknowledge the Department of Environment, Government of India, Ministry of Environment and Forests for grants-in-aid.

REFERENCES

1. Williams, R.J.P. :
Eur. J. Biochem. 150[2], 231, [1985].

2. Farias, R.N., Goldemberg, A.L. and Trucco, R.E. :
Arch. Biochem. Biophys. 139, 38 [1970].

3. Schatzmann, H.J. and Vincenzi, F.F. :
J. Physiol, [London] 201, 369 [1969].

4. Susheela, A.K., Sharma, Y.D., Jha, M., Singh, M., Jagannath, B. and Jain, S.K. :
Fluoride, 15[4], 173, [1982].

5. Susheela, A.K. and Jain, S.K. :
In : Studies in Environmental Science, Fluoride Research 1985, Vol. 27 [H. Tsunoda and M.H. Yu, eds.], Elsevier, Amsterdam, pp 231-239, [1986].

6. Susheela, A.K. and Jain, S.K. :
IRCS, 11, 14, [1983].

7. Shimamoto, N., Goto, N. and Hirata, M. :
Nippon Yakurigaku Zasshi, 80[2] : 137, [1982].

8. Rich, C. and Ensinck, J. :
Nature [London]; 191, 184, [1961].

9. Reddy, G.S. and Narasinga Rao, B.S. :
Metabolism; 20, 650, [1971].

10. Ophaug, R.H. and Singer, L. :
J. Nutr., 106, 771, [1976].

11. Bond, G.H. and Green, J.W. :
Biochem. Biophys. Acta. 241, 393, [1971].

12. Fiske, C.H. and Subba Row, Y. :
J. Biol. Chem. 61, 375, [1925].

13. Bond, G.H. and Clough, D.L. :
Biochem. Biophys. Acta. 323, 592, [1973].

14. Linhardt, K. and Walter, K. :
In : Methods of enzymatic analysis [H.U. Bergmeyer, ed.] 2nd edition, pp 781, Acad. Press, New York, [1965].

15. Prasad, A.S. :
In : Trace elements and iron in human metabolism. Plenum Medical Book Co., New York, [1978].

16. Barker, D.H., Rencher, A.C., Mittal, B.M., Shanbhag, S.V., Sharma, V.N. and Sharma, L.S. :
In : Trace substances in environmental health [D.D. Hemphil, ed.] Vol. X, Univ. of Missouri, Columbia, [1976].

17. Hall, L.L., Smith, F.A. and Hodge, H.C. :
Proc. Soc. Exp. Biol. Med. 139, 1007, [1972].

18. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. :
J. Biol. Chem. 193, 263, [1961].

19. Vincenzi, F.F. :
In : Cellular mechanism for calcium transfer and homeostasis, ed. G. Nichals, Jr. and R.H. Wasserman, New York, Academic Press [1971].

20. Woodin, S.M. :
In : Biological bases of Medicine, Vol. 2, ed. E.E. Bittar, pp 373, Acad. Press, New York, [1968].

21. Campbell, A.K., Daw, R.A., Hallet, M.B. and Luzio, J.P. :
Biochem. J. 194, 551, [1981].

22. Penniston, J.T. :
Ann. NY. Acad. Sci.; 402, 296, [1982].

23. Suketa, Y., Mikami, E., Sato, Y., Hayashi, M. and Yamamoto, T. :
J. Toxicol. Environ. Health 2, 301, [1976].

(Received in UK 16 January 1987)