CHEMICAL PROFILE OF PLASMA IN FLUORIDE TOXICITY II TOTAL PROTEIN-BOUND HEXOSE AND SEROMUCOID FRACTION OF RABBIT PLASMA

by

A.K. Susheela and Y.D. Sharma
New Delhi, India

SUMMARY: Two groups of rabbits were given NaF intragastrically in doses of 10 and 50 mg/kg body weight/day respectively. Blood was drawn from both groups on day 10, 25, 50, 75, 90 and 125 after administration of NaF. Total protein-bound hexose and seromucoid fraction (as its hexose and protein contents) were determined spectrophotometrically. The effect of the two different doses of NaF on the total protein-bound hexose and seromucoid fraction in the rabbit plasma was followed up. With low doses glycoprotein levels were decreased in the blood circulation. Inhibition of glycoprotein biosynthesis and absence of certain individual glycoproteins are likely to result in low plasma glycoprotein levels. Besides, in the process of new bone formation known to occur in fluoride poisoning, certain glycoproteins tend to combine with calcium which leads to its deposition in tissues and lowers the glycoprotein levels in plasma. But high doses of NaF enhanced the glycoproteins in plasma. Thus the activity of certain lysosomal enzymes like neuraminidase is likely to be inhibited by fluoride ions reducing glycoprotein catabolism and leading to high levels of plasma glycoprotein. At the same time, the

From the Fluorosis Research Laboratory, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

increased concentration of certain individual glycoproteins or the addition of newly synthesized glycoproteins due to tissue injury may result in elevation of plasma glycoprotein in the circulation.

Introduction

Glycoproteins, the proteins conjugated with carbohydrates as prosthetic groups, covalently linked to their protein part, carry out numerous biological functions. Plasma contains a vast number of proteins; most of them except albumin are included in glycoproteins. Schultze and Heremans (1) have listed 31 glycoproteins in normal human plasma. In pathological states, the number and concentration of glycoproteins are known to be altered by the addition of newly synthesized glycoproteins. The first report on glycoproteins in cancer appeared in 1930. Since then glycoproteins have been studied in various pathological conditions. Winzler (2) cited 21 different pathological conditions in which protein bound carbohydrate was found to be significantly increased. Circulating glycoproteins were found to be deficient (3-5) in other pathological conditions.

Several authors have reported a correlation of total protein bound hexose of plasma with the level of typical acute phase reactants, namely fibrinogen, heptoglobin or seromucoid fraction (6, 7, 8). Acute phase proteins (particularly the seromucoid fraction) are of considerable interest because their changes in plasma are regarded as a sensitive (although rather nonspecific) test for diagnostic and prodiagnostic assessments. In this regard we have reported the effect of low and high doses of NaF on rabbit plasma fibrinogen, an acute phase protein (9). The changes in the seromucoid fraction and total serum glycoproteins in human fluorosis have also been reported (10). Winzler (11) for the first time approached a finer characterization of plasma glycoproteins by preparing and estimating the seromucoid fraction.

For a meaningful analysis of the results, studies have been extended to an experimental animal model. The present communication describes the effect of NaF on total protein-bound hexose and seromucoid levels of plasma of rabbits given NaF at varying time intervals.

Material and Methods

Two batches comprised of 3 and 8 healthy rabbits weighing 0.9 to 1.5 kg were given 50 and 10 mg NaF/kg body weight respectively through the intragastric route at 24 hour intervals. Six control animals maintained under the same laboratory conditions received no NaF. Blood samples were drawn on day 10, 25, 50, 75, 90 and 125 through ocular vein puncture and plasma was separated out.

Total Protein-bound Hexose: In order to estimate the total protein bound hexose, the plasma was treated with 95% ethanol. The precipitate was separated and dissolved in 0.1N NaOH. A known aliquot of the sample was treated with orcinol at 80°C according to the Winzler (11) method.

Figure 1

Effect of NaF on Total Protein-Bound Hexose of Rabbit Plasma

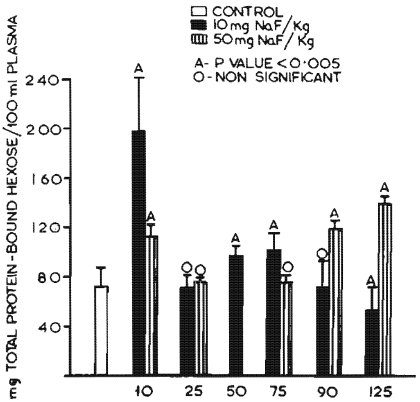
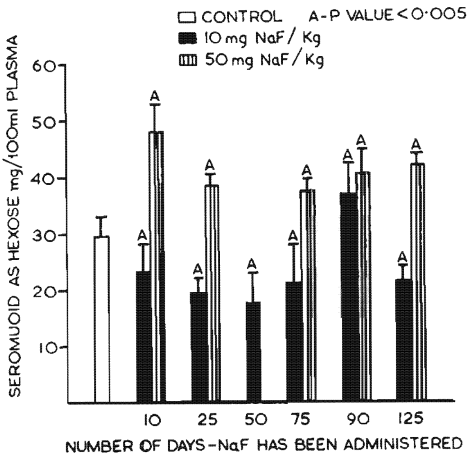


Figure 2

Effect of NaF on Seromucoid Fraction (as Hexose) of Rabbit Plasma



An equimolar mixture of galactose and mannose was used as standard. The optical density measurements were carried out on a Zeiss spectrophotometer (PMQII). The results are expressed as mg percent.

Seromucoid Fraction: This fraction was measured in terms of its hexose and protein contents. Plasma proteins other than seromucoid were precipitated out with 1.8 M perchloric acid. The precipitate thus obtained was discarded. The supernatant was treated with 5% phosphotungstic acid. The precipitates were dissolved in 0.1N NaOH. Hexose and protein contents were measured by the Winzler (11) method using orcinol and by the Lowry method (12) respectively. Results are expressed as mg%.

Results

The results obtained for total protein-bound hexose and seromucoid fraction from normal and NaF treated rabbits are shown in the diagrams.

Total Protein-bound Hexose: The total protein-bound hexose of plasma of rabbits treated with low dose of 10 mg/NaF/kg body weight for 10, 25, 50, 75, 90 and 125 days are shown in Fig. 1. The total protein-bound hexose content on day 10 after NaF ingestion, has risen compared to normal. The increase, although significant at P value < 0.005, is not of long duration. By day 25, the total protein-bound hexose is reduced to normal levels. Repeated administration of NaF has induced a gradual increase in total protein-bound hexose, from day 25 to 75, and a gradual decline through day 90 to 125. When the result of a dose of 10 mg NaF is compared with the result of 50 mg NaF, an increase on day 10 at the initial phase (signification at P value < 0.0005) is followed on day 25 by a reduction to normal levels. The duration of normal levels up to day 75 was followed by a gradual increase in total protein-bound hexose from day 90

to 125 (significant at P value < 0.0005). The results obtained for total protein-bound hexose from large doses of NaF on day 125 are in contrast to those obtained from low doses of NaF namely an increase of the protein-bound hexose of 93.56% compared to a decrease of 22.34% for the low doses as related to normal.

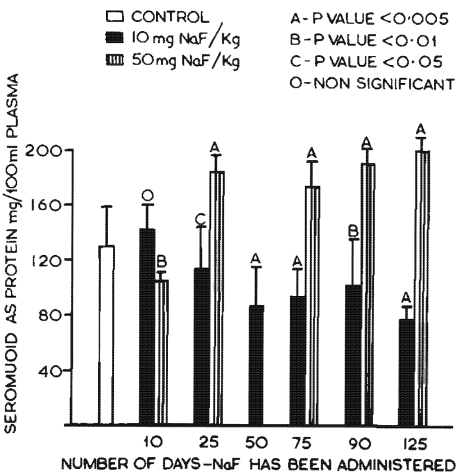
Seromucoid Fraction: The seromucoid fraction was measured in terms of its hexose and protein contents in normal plasma and that of the NaF rabbits. The results obtained for seromucoid (both as hexose and protein) on day 10, 25, 50, 75, 90 and 125 after ingestion of NaF are shown in Fig. 2, 3. The results obtained as hexose and protein which represents the seromucoid fraction are described separately.

Hexose: Seromucoid levels measured as hexose in low doses (10 mg/NaF/kg body weight) are depicted in Fig. 2. It can be seen that seromucoid levels declined on day 10 after NaF ingestion. The reduction persisted up to day 50; the results are significant at P value < 0.005.

Subsequently levels rose gradually through day 75 up to 90, buy by day 125 a reduction was significant (P < 0.005). Seromucoid levels (measured as hexose) in rabbits on 50 mg/NaF/kg body weight were high compared to normal. The increase in hexose content, through the experimental period remained significantly high (P < 0.005). The response of different doses of NaF to seromucoid measured as hexose is of interest. On day 90, the seromucoid levels both for low and high doses were significantly above normal, whereas during the remaining phases, they were reduced by the low doses and increased by the high doses of NaF compared to normal.

Figure 3

Effect of NaF on Seromucoid Fraction (as Protein) of Plasma



Protein: The seromucoid levels measured as protein in low doses of NaF are shown in Fig. 3. On day 10 after NaF ingestion, seromucoid is not significantly increased. However on day 25, 50, 75, 90 and 125 the seromucoid is reduced significantly at P < 0.05, P < 0.005, P < 0.005, P < 0.01 and P < 0.005 respectively.

Seromucoid levels associated with 50 mg/NaF/kg body weight contrast with those from low doses. On day 10 after ingestion of NaF, there was a reduction ($P < 0.01$) in seromucoid levels, followed by a gradual increase with repeated administration of NaF from day 25 to 125. This increase was significant at P value < 0.005.

The ratio of carbohydrate to protein in the seromucoid fraction was 0.23 in normal rabbit plasma. However it decreased with low doses of NaF to 0.16, 0.21 and 0.22 on day 10 to 25, 50 and 75 respectively whereas on day 90 and 125 with low doses of NaF the ratio increased to 0.36 and 0.27 respectively. On administration of high doses of NaF, the ratio was found to be slightly decreased to 0.21 throughout the treatment except on day 10, when the ratio was increased to 0.46.

Discussion

The increase in total protein-bound hexose in rabbit plasma on day 10 at the initial phase of fluoride intoxication with both high and low doses of NaF may be due to increased concentration of some of the glycoproteins present in the α-globulin fraction, which act as antibodies if we consider NaF as a foreign material. Various groups of investigators (13, 14) have demonstrated electrophoretically an increase in the concentration of γ-globulin in fluoride toxicity.

The normalization of the reduced total protein-bound hexose on day 25 with both low and high doses of NaF may be due to compensatory mechanisms. But the increase on day 50 and 75 from low doses of NaF may be related to the addition of some newly synthesized glycoproteins to the blood circulation (2, 15). On further ingestion of low doses of NaF, the increase was followed by a gradual reduction in the total protein-bound hexose, from day 90 to 125. This reduction can be explained as follows:

(1). The concentration of some of the individual glycoproteins in plasma may be decreased due to low levels of these components in the tissues/organs where they are synthesized (16). These low levels (16) may also be due to the retention of some of the acute-phase proteins such as fibrinogen and heptoglobin, in extravascular pool, where they participate in tissue repair (9, 17). Our previous findings which showed low levels of the seromucoid fraction in blood serum of fluorosis patients, also support this view (10). Some of the glycoproteins are likely to be deposited in tissues by forming calcium bridges. During fluoride intoxication, the plasma total protein-bound hexose is therefore reduced (10, 18-20).

(2). It is also possible that the glycoprotein biosynthesis - may be reduced during fluoride toxicity. It has been reported that fluorine ions interfere with protein biosynthesis (21). Glycoproteins are mainly synthesized in the liver and liver function is impaired in fluoride toxicity (13, 14, 21), resulting in reduction of glycoprotein levels in blood.

(3). The catabolism of glycoproteins may be increased by low doses of NaF due to activation of certain lysosomal glycosidic enzymes. In this connection Cimasoni stated (22) that the fluoride ion in low concentration can stimulate certain enzymes. Therefore, it is presumed that with low doses of NaF the increased lysosomal neuraminidase activity removes sialic acid from the terminal position of the oligosaccharide chains, producing asialoglycoproteins. These asialoglycoprotein molecules are susceptible to the hepatocytes causing their removal from circulation (23-26).

With large doses of NaF, total protein-bound hexose levels in plasma increase on day 90 and 125. This increase may be due to:

(a). Increased concentration of some of the individual glycoproteins such as fibrinogen (9).

(b). Addition of newly synthesized glycoproteins to the blood circulation. During fluoride toxicity, tissue damage takes place which accounts for an elevation of glycoprotein levels in the blood (13, 14, 27). Under various pathological conditions, several investigators have suggested that the humoral factor, released from injured tissues, stimulates the glycoprotein biosynthesis (28).

(c). In high doses, fluoride ions have an inhibitory effect on lysosomal enzymes (22), resulting in a decreased catabolism of glycoproteins. Neuraminidase activity can be inhibited by large doses of NaF. It is also known that lysosomal neuraminidase activity can be inhibited by Na^+ ions (26).

During the present investigation the seromucoid levels from low doses of NaF were found to be decreased (both as hexose and protein) throughout the experimental period except on day 90, when the seromucoid measured as hexose was increased compared to the normal. The seromucoid fraction exists in γ-globulins fraction of serum/plasma on electrophoresis (29). Yu et al. (14) have demonstrated electrophoretically the reduced concentration of γ-globulins in chicken blood after fluoride ingestion. It has been suggested that the fluoride ion can induce changes in the membrane permeability (30-32). Changes in serum proteins and enzyme concentration in blood circulation ensue. Various investigators have shown that the seromucoid fraction is heterogenous, containing a number of proteins and glycoproteins whose proportions may vary independently under pathological conditions (17).

High doses of NaF responded differently to seromucoid levels which increased, both as hexose and protein, throughout the experimental phase except on day 10, when the seromucoid as protein declined. This general increase in the levels may be due to reduction in catabolism, because fluoride in high doses inhibits the lysosomal enzyme activity (22). The increase in this acute-phase protein biosynthesis also cannot be entirely ruled out because tissue damage can occur from large doses of fluoride, which induce the biosynthesis of glycoproteins. Fibrinogen levels are also increased by large doses of NaF in rabbit plasma (9).

The ratio of carbohydrate to protein in the seromucoid fraction is fairly constant in most normal and pathological sera. During the present study, this ratio was altered on day 10, the initial phase of fluoride intoxication. The values obtained on day 10 from low and high doses of NaF, contrast with each other. From low doses of NaF this ratio showed a reduction whereas from large doses, it was distinctly elevated. This ratio was also elevated on day 90 and 125 from low doses of NaF. The other values remained normal or slightly below normal. The most likely explanation for this phenomenon is that, in these conditions, the proportion of carbohydrate-poor and carbohydrate-rich components in the seromucoid fraction is altered, although the possibility of synthesis of modified glycoprotein molecules cannot be entirely ruled out (28). In certain pathological conditions, such as liver diseases, renal disease and rheumatoid arthritis (11), the ratio of carbohydrate to protein in seromucoid is elevated but in diabetes (33) or silicosis (34) this ratio is reduced.

It is concluded that the two different doses of NaF respond differently to the total protein-bound hexose and seromucoid fractions. Low doses of NaF decrease glycoproteins in the circulation. Inhibition of glycoprotein biosynthesis and the absence of certain individual glycoproteins ensue from low levels of plasma glycoproteins. Besides, new bone formation which is known to occur in fluoride intoxication is likely to induce glycoproteins to combine with calcium ions and thus eliminate calcium from circulation. But with high doses of NaF, the inhibition of certain lysosomal enzymes like neuraminidase by fluoride ions may reduce the glycoprotein catabolism resulting in increased levels of glycoproteins in blood plasma. At the same time, the increased concentration of certain individual glycoproteins or addition of newly synthesized glycoproteins due to tissue injury, should be considered which results in high levels of plasma glycoproteins in blood circulation.

Acknowledgements

This work was supported by substantial grants-in-aid from the Department of Science and Technology (DST) (Government of India) and the Indian Council of Medical Research. One of us (Y.D.S.) acknowledges the DST for a Research Associateship, during the tenure of which the investigations were carried out.

Bibliography

1. Schultze, H.E., and Heremans, J.F.: Molecular Biology of Human Proteins. Vol. 1, Elsevier, Amsterdam, 1966.
2. Winzler, R.J.: Glycoproteins in Disease. Ch.: XII. In Glycoproteins of Blood Cells and Plasma. Ed. Jamieson, G.A. and Greenwalt, T.J., 1971, 204-218.
3. Schmid, K., Burke, J.F., Derbay Saschs, M., and Tokita, K.: Sialic Acid Deficient α_1 - Acid Glycoprotein Produced in Certain Pathological States. Nature (London), 204:75-76, 1964.
4. Eriksson, S.: Pulmonary Emphysema and Alpha$_1$ - Antitrypsin Deficiency. Acta Med. Scand., 175:197-205, 1964.

5. Talamo, R.C., Allen, J.D., Kahan, M.G., and Auster, K.F.: Hereditary α_1 - Antitrypsin Deficiency. New Eng. J. Med., 278:345-351, 1968.
6. Koj, A., and McFarlane, A.S.: Effect of Endotoxin on Plasma Albumin and Fibrinogen Synthesis Rates in Rabbits as Measured by the [^{14}C] Carbonate Method. Biochem. J., 108:137-146, 1968.
7. Weimer, H.E., and Coggshall, V.: Divergent Responses of Serum Glycoprotein Fractions to Tissue Injury in Adrenalectomized Rats. Can. J. Physiol. Pharmacol., 45:767-775, 1967.
8. Winzler, R.J.: Metabolism of Glycoproteins. Clin. Chem., 11:339-347, 1965.
9. Susheela, A.K., and Sharma, Y.D.: Chemical Profile of Blood in Fluoride Toxicity III. Plasma Fibrinogen Levels in Rabbit. In Press.
10. Susheela, A.K., Sharma, Y.D., Jha, M., RajLaxmi, K., and Rammohan Rao, N.V.: Chemical Profile of Serum in Fluorosis and Fluoride Toxicity. I. Total Glycoprotein and Seromucoid Fraction of Human Sera. In Press.
11. Winzler, R.J.: Determination of Serum Glycoproteins. In Methods of Biochemical Analysis. Ed. D. Glick, Vol.II Interscience Publisher, Inc. New York, 1955, pp. 279-311.
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein Measurements with Folin Phenol Reagent. J. Biol. Chem., 193:265-275, 1951.
13. Kaur, R., Singh, P., and Makhni, S.S.: Longterm Effects of Fluoride Administration - An Experimental Study II, Effect on Serum Proteins. Fluoride, 11:25-28, 1978.
14. Yu, M.H., Stoehr, M.P., and Driver, C.J.: Electrophoresis of Serum Proteins in Growing Chicks Fed a Diet Supplemented with NaF. Fluoride, 13:20-24, 1980.
15. Bekski, J.G., Macbeth, R.A.L., and Bice, S.: The Metabolism of Plasma Glycoproteins II. Studies on the Rate of Incorporation of Glucosamine-1-^{14}C into Protein-bound Hexosamine in the Rat Bearing Walker 256 Carcinoma. Cancer Res., 26:2307-2315, 1966.
16. Bogin, E., Abrams, M., Avidar, Y., and Israeli, B.: Effect of Fluoride on Enzymes from Serum, Liver, Kidney, Skeletal and Heart Muscles of Mice. Fluoride, 9:42-46, 1976.
17. Koj, A.: The Ciba Foundation Symposium: Energy Metabolism in Trauma. Eds. R. Porter and J. Knight., Churchill, London, 1970, p. 79.
18. William, P.A., and Peacocke, A.R.: Binding of Calcium and Yttrium to a Glycoprotein from Bovine Cortical Bone. Biochem. J., 105:1177-1185, 1967.
19. Chipperfield, A.R.: Calcium Ion Binding by Bone Mucosubstances. Biochem. J., 118 (No. 1-3): 1970, p. 36 P.
20. Bernard, B. De, Stagni, N., Vitter, F., and Zanetti, M.: Role of Ca^{++} Binding Glycoprotein in the Process of Calcification. In Calcium Binding Proteins and Calcium Function, Ed. R.H. Wasserman et al. Elsevier, North-Holland, New York 1977.
21. Kathpalia, A., Susheela, A.K.: Effect of Sodium Fluoride on Tissue Protein in Rabbits. Fluoride, 11:125-129, 1978.
22. Cimasoni, G.: Fluoride and Enzymes. In Fluoride in Medicine, Ed. Vischer, T.L. Hans Huber, Bern, Stuttgart, Vienna 1970, pp. 14-26.

23. Hickman, R., Ashwell, G., Morell, A.G., Van Den Hamer, C.J.A., Scheinberg, I.H.: Physical and Chemical Studies on Ceruloplasmin VIII Preparation of N-acetyl-neuraminic Acid - 1 - C^{14} - labelled Ceruloplasmin. J. Biol. Chem. 245:759-766, 1970.
24. Van Den Hamer, C.J.A., Morell, A.G., Scheinberg, I.H., Hickman, J. and Ashwell, G.: Physical and Chemical Properties of Ceruloplasmin IX. The Role of Galactosyl Residues in the Clearance of Ceruloplasmin from Circulation. J. Biol. Chem., 245:4397-4402, 1970.
25. Morell, A.G., Greggriadis, G., Scheinberg, I.H., Hickman, J., and Ashwell, G.: The Role of Sialic Acid in Determining the Survival of Glycoproteins in the Circulation. J. Biol. Chem. 246:1461-1467, 1971.
26. Vimal Patel, G.A., and Tappel, A.L.: Glycosidases in Glycoprotein Catabolism, In Glycoproteins of Blood Cells and Plasma. Ed. Jameson, G.A. and Greenwalt, T.J., 1971, pp. 133-163.
27. Jolly, S.S., Sharma, O.P., Garg, G. and Sharma, R.: Kidney Changes and Kidney Stones in Endemic Fluorosis. Fluoride, 13:10-16, 1980.
28. Koj, A.: Acute Phase Reactants. In Structure and Function of Plasma Proteins.I.Ed. Allison, A.C. Plenum Press, London, 1974 pp. 73-131.
29. Weiss, J.E., Bradley, W.P., Blasco, A.P., Alexander, J.C. Jr., Silverman, N.A.: Serum Protein-bound Carbohydrates and Other Glycoprotein Assays as Indicators of Tumor Burden. U.S. Government Publication, 1979.
30. Furguson, D.B.: Effects of Low Doses of Fluoride on Serum Proteins and a Serum Enzyme in Man. Nature, New Biol. 231:159-160, 1971.
31. Riekstneice, E., Meyers, H.M. and Glass, L.E.: In Vivo Effects of Sodium Fluoride on Serum Proteins and Enzymes as Studied with Starch Gel Electrophoresis. Arch. Oral. Biol., 10:107-118, 1965.
32. Zebrowski, E.J., and Suttie, J.W.: Glucose Oxidation and Glycogen Metabolism in Fluoride-Fed Rats. J. Nutr., 88:267-271, 1966.
33. Sarnecka-Keller, M., Wozniczka, K., and Ciba, T.: Proc. XX Conference of the Diabetologic Section of the Polish Soc. of Int. Med., Krakow, 1971.
34. Sugar, E.A.: The Carbohydrate Content of Serum Mucoprotein. Comparative Study of Normal and Silicotic Sera. Clin. Chem. Acta., 8:347-350, 1967.

****

FLUORIDE BRIEFS

When NaF and Na_2PO_3F are added to rat hepatocytes, lactate is decreased and production of glucose, 3 and 2-phosphoglycerate is increased. The activity of some of the glycolytic enzymes may be inhibited in the presence of NaF and Na_2PO_3F.

Shahed, A.R., Miller, A.R., and Allmann, D.W.: Influence of NaF and Na_2PO_3F (MFP) on Glucose Metabolism in Rat Hepatocytes. Biochem. Biophys. Res. Communications, 91:583-591, 1979.

****