0148-6071/83/0703-0293$02.00/0
JOURNAL OF PARENTERAL AND ENTERAL NUTRITION
Vol. 7, No. 3
Printed in U.S.A.

Reversal of Biochemical and Functional Abnormalities in Erythrocytes Secondary to Selenium Deficiency

SUSAN S. BAKER, M.D., PH.D., WALTER W. K. KING, M.D., LUC MICHEL, M.D., WILLIAM C. WOOD, M.D., RONALD A. MALT, M.D., AND HARVEY J. COHEN, M.D., PH.D.

From the Surgical Services, Massachusetts General Hospital, and the Department of Surgery, Harvard Medical School; the Division of Hematology, and the Division of Gastroenterology, Children's Hospital Medical Center, Boston, Massachusetts, and the Department of Food Science and Nutrition, MIT, Cambridge, Massachusetts

ABSTRACT. A patient with multiple enterocutaneous fistulae on total parenteral nutrition for 14 months developed low erythrocyte selenium and low erythrocyte glutathione peroxidase. Erythrocyte hexose monophosphate shunt activity stimulated with an H_2O_2 generating system was approximately one-fourth that of control. Hexose monophosphate shunt activity stimulated with methylene blue showed little difference between patient and control. With selenium supplementation erythrocyte selenium, glutathione peroxidase, and hexose monophosphate shunt activity became normal. Thus, the biochemical and functional consequences of selenium deficiency can be corrected with selenium supplementation.

Selenium is the metallocofactor for the enzyme glutathione peroxidase (GSHPx), which functions to metabolize H_2O_2.[1] In the absence of selenium GSHPx is not active.[2] Until recently, selenium deficiency disease has not been reported in humans. However, Chinese investigators have shown that Keshan disease, a cardiomyopathy endemic in selenium-poor areas in China is associated with low serum and hair selenium.[3] This cardiomyopathy affects children and women of child-bearing age, and occurs with seasonal variation. It is prevented but not cured with selenium supplementation.[4]

Low erythrocyte selenium and GSHPx have been reported in patients maintained on total parenteral nutrition (TPN).[5,6] We have recently studied a 43-yr-old man maintained on TPN for 2 yr, who developed a cardiomyopathy of sudden onset which was unresponsive to medical management. He was shown to have low erythrocyte selenium and GSHPx. Myocardial selenium and GSHPx were low, but glutathione reductase and glucose-6-phosphate dehydrogenase were normal. The histopathologic features of his heart were comparable to those found in Keshan disease.[7]

We now present a patient maintained on TPN for 14 months, who developed selenium deficiency associated with both biochemical and functional abnormalities in his erythrocytes. The selenium deficiency and the biochemical and functional abnormalities were reversed with selenium supplementation.

CASE REPORT

A 39-yr-old man with a diagnosis of pancreatitis complicated by enterocutaneous fistulae requiring multiple surgical procedures was maintained on TPN for 14 months. The patient did not complain of muscle pain or tenderness and when specifically asked denied myalgia. There was no clinical evidence of congestive heart failure or arrhythmia. In addition, there were no EKG changes consistent with congestive heart failure or arrhythmia. The patient was found to be deficient in erythrocyte selenium and GSHPx. His hemoglobin was 11.1 g/100 ml, hematocrit 33% with mean corpuscular hemoglobin of 28 μg, mean corpuscular hemoglobin concentration of 33%, and mean corpuscular volume of 84 μ^3. There was no evidence for hemolysis such as an increased reticulocyte with a decreased hematocrit or increased unconjugated bilirubin. The patient was supplemented with commercially available selenium tablets, 100 μg four times a day for 7 days, then 25 μg four times a day for 4 months. He was restudied after 4 months. The patient was not receiving oxidant drugs at the times he was studied. After 2 months on supplementation it was interrupted for a surgical procedure, then reinitiated.

METHODS

Selenium was determined fluorometrically.[8] GSHPx was assayed according to Beutler.[9] Hexose monophosphate shunt (HMPS) activity was measured using 0.05 U/ml L-amino acid oxidase plus 5.0 mM L-leucine and 0.1 mM methylene blue.[10] A cyanide ascorbate test was performed by the method of Jacob and Jandl.[11]

RESULTS

Table I shows that when initially studied, the patient had low erythrocyte selenium and GSHPx activity. HMPS activity in patient erythrocytes stimulated with L-amino acid oxidase and L-leucine was one-fourth of the control. HMPS activity in patient erythrocytes when stimulated with methylene blue was the same as the control. A cyanide ascorbate test was abnormal. After 4 months of selenium supplementation these values became normal.

Received for publication, April 7, 1982.
Accepted for publication, November 11, 1982.
Reprint requests to: Dr. Susan S. Baker, Department of Gastroenterology and Nutrition, Children's Hospital Medical Center, 300 Longwood Avenue, Boston, MA 02215.

TABLE I
Biochemical studies on erythrocytes in a patient with selenium deficiency before and after selenium supplementation

Erythrocytes	Before selenium supplementation	After selenium supplementation
Hematocrit (%)	33	34
Selenium (ng/mg hemoglobin)	0.040[a]	0.63
GSHP × (IU/g hemoglobin)	2.86[b]	20.4[b]
HMPS (cpm/g hemoglobin)		
L-Amino acid oxidase plus L-leucine	0.7×10^3 (3.3×10^3)[c]	3.5×10^3 (2.0×10^3)[c]
Methylene blue	2.3×10^3 (2.0×10^3)[c]	1.8×10^3 (2.1×10^3)[c]
Cyanide ascorbate	Abnormal	Normal

[a] Normal levels for adult males are 0.7 ± 0.2 ng/mg hemoglobin.
[b] Normal GSHPx levels in our lab are 24 ± 5 IU/g hemoglobin.
[c] Figures in parentheses are normal subjects assayed at the same time as the patient.

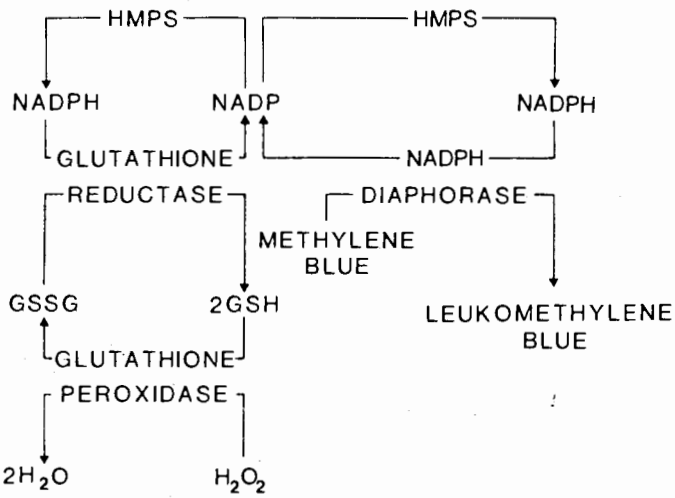

FIG. 1. Stimulation of the hexose monophosphate shunt by the glutathione cycle and methylene blue. NADP, nicotinamide adenine dinucleotide; NADPH, reduced NADP; GSSG, oxidized glutathione; GSH, reduced glutathione.

DISCUSSION

In the erythrocyte, selenium is necessary for GSHPx activity. As can be seen in Figure 1, GSHPx functions to reduce H_2O_2 by the oxidation of reduced glutathione to the oxidized form. Reduced glutathione is regenerated from oxidized glutathione by glutathione reductase which oxidizes NADPH to NADP. NADPH is regenerated through the HMPS.

The cyanide ascorbate test measures the ability of erythrocytes to metabolize H_2O_2 generated through the coupled oxidation of ascorbate in the presence of oxyhemoglobin. Catalase activity is inhibited by cyanide.

If glutathione peroxidase is limiting, erythrocytes cannot metabolize H_2O_2. In the presence of an H_2O_2 generating system (L-amino acid oxidase and L-leucine) normal erythrocytes are able to metabolize H_2O_2 through the glutathione cycle, and the activity of the HMPS is increased. In this patient's GSHPx-deficient erythrocytes, H_2O_2 was not metabolized as efficiently and the activity of the HMPS was not increased as much as the control. H_2O_2 accumulated and caused an abnormal cyanide ascorbate test. The abnormal cyanide ascorbate test indicates that the patient's erythrocytes could not respond to an oxidative stress. Methylene blue stimulation of HMPS was not altered in the patient's erythrocytes. This can be explained by the ability of methylene blue to increase the HMPS by direct enzymatic oxidation of NADPH (Fig. 1), thus avoiding the glutathione cycle, and demonstrating that the HMPS itself is intact. Despite these biochemical and functional abnormalities, there was no evidence for hemolysis. While this patient exhibited no hemolysis, it is possible that oxidant drugs may precipitate hemolytic episodes.

After treatment with selenium, erythrocyte selenium and GSHPx increased to normal. The response of the HMPS to an H_2O_2 generating system became normal as did the cyanide ascorbate test. Although not studied directly, this return to normal is probably due to the production of selenium-replete erythrocytes which replaced the deficient ones.

This demonstrates that GSHPx deficiency can be associated with functional abnormalities of erythrocyte metabolism and these abnormalities can be corrected with selenium repletion. It also suggests that selenium should be added to TPN solutions, particularly for patients who need long-term parenteral nutrition.

ACKNOWLEDGMENTS

Supported in part by the Stanley Thomas Johnson Foundation, Grant 5T32-AMO-7070-05 from the National Institutes of Health, and Grant CA-26506 from the National Cancer Institute. H.J.C. is the recipient of Grant AI-0031 from the National Institutes of Allergy and Infectious Disease.

The authors thank Dr. Orville Levander, of the Beltsville Human Nutrition Research Center, United States Department of Agriculture, Beltsville, MD, for performing erythrocyte selenium determinations; Ms. Jeanne M. Wilson, and Ms. M. Teresa Holland, of the Nutritional Support Unit, Massachusetts General Hospital for their help and encouragement; Orah Platt, MD, of Children's Hospital Medical Center for performing the cyanide ascorbate test; and Ms. Sally Burke for secretarial assistance.

REFERENCES

1. Rotruck JT, Pope AL, Ganther HE, Swanson AS, Hafeman DG, Hoekstra WG: Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588-590, 1973
2. Forstrom JW, Zahowski JJ, Tappel AL: Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry 17:2639-2644, 1978
3. Keshan Disease Research Group of the Chinese Academy of Medical Sciences: epidemiologic studies on the etiologic relationship on selenium and Keshan disease. Chinese Med J 92:477-482, 1979
4. Keshan Disease Research Group of the Chinese Academy of Medical Sciences: observations on the effect of sodium selenite in prevention of Keshan disease. Chinese Med J 92:471-476, 1979
5. Van Rij AM, Thomson CO, McKenzie JM, Robinson MF: Selenium deficiency in total parenteral nutrition. Am J Clin Nutr 32:2076-2085, 1979
6. Phillips GD, Garnys VP: Trace elements balance in adults receiving parenteral nutrition: preliminary data. JPEN 5:11-14, 1981

7. Johnson RA, Baker SS, Fallon JT, et al: An occidental case of cardiomyopathy in selenium deficiency. N Engl J Med 304:1210–1212, 1981
8. Hoffman I, Westerby RJ, Hiiroglou M: Precise fluorometric microdetermination of selenium on agricultural materials. J Assoc Anal Chem 51:1039–1042, 1968
9. Beutler E: Red Cell Metabolism. A Manual of Biochemical Methods. Grune & Stratton, New York, 1975
10. Stossel TP, Murad F, Mason RJ, Vaughn M: Regulation of glycogen metabolism in polymorphonuclear leukocytes. J Biol Chem 245:6228–6234, 1970
11. Jacob HS, Jandl JS: A simple visual screening test for glucose-6-phosphate dehydrogenase deficiency employing ascorbate and cyanide. N Engl J Med 274:1162–1167, 1966