REDUCED ZINC IN PERIPHERAL BLOOD CELLS FROM PATIENTS WITH INFLAMMATORY CONNECTIVE TISSUE DISEASES

KARIN L. G. SVENSON, ROGER HÄLLGREN, ERLAND JOHANSSON, and ULF LINDH

Department of Internal Medicine, University Hospital
Gustaf Werner Institute, Department of Physical Biology
Uppsala, Sweden

Abstract—By the use of the nuclear microprobe technique, the concentrations of zinc in isolated erythrocytes, platelets, and granulocytes were measured in patients with rheumatoid arthritis, other inflammatory arthritides, and scleroderma. Markedly reduced cellular zinc values were found compared to those measured in healthy subjects. No relation was found to inflammatory activity or disease duration. Plasma zinc was reduced in the majority of the patients and was negatively correlated to the inflammatory activity estimated by ESR and serum orosomucoid. No relation was found between total zinc values in plasma or cells or disease duration. Corticosteroid therapy was instituted in a number of the patients with inflammatory arthritides and induced a significant elevation of total zinc in all cell types, although normalization was not achieved. Plasma zinc values remained unchanged during the treatment.

INTRODUCTION

There is a basic requirement for zinc (Zn) in order for cells to function. Zinc is involved in nearly all aspects of cellular metabolism and plays a key role in numerous essential processes: protein synthesis, DNA and RNA metabolism, and carbohydrate and lipid metabolism (1). Zinc plays a crucial role in maintaining cell membrane structure and function (2) and influences immune response (3) complement system (4), lysosomal enzyme release (5), and macrophage function (6). Based on these and other zinc-dependent effects, it is not surprising that considerable attention has been paid to zinc as an element of importance in inflammatory diseases.

1 Supported by grants from the Swedish Medical Research Council, the Swedish Natural Science Research Council, and Pharmacia A.B.
In most studies on rheumatoid arthritis plasma zinc is reported to be reduced, but it is uncertain whether or not this really reflects a true zinc deficiency of importance for the disease process. Clinical improvement in patients with rheumatoid arthritis treated with zinc sulfate has been reported (7), but other studies have failed to confirm such an effect (8, 9). Total body zinc is not accurately reflected by serum or plasma levels, since zinc mainly exists in the intracellular compartment (10). In this study we have measured the zinc concentrations in isolated peripheral blood cells: erythrocytes, granulocytes, and platelets, from a group of patients with rheumatoid arthritis, other inflammatory arthritides, and scleroderma. The data obtained were related to the duration and activity of the disease, and the effect of anti-inflammatory treatment was elucidated.

MATERIALS AND METHODS

Patients. Peripheral blood was collected from 24 inpatients with inflammatory connective tissue diseases: rheumatoid arthritis (RA) (N = 11), ankylosing pelvospndylitis (N = 3), psoriasis arthropathy (N = 3), Reiter's disease (N = 1), postinfectious arthritis (N = 1), and scleroderma (N = 5). The patients with RA all had classical or definite disease according to the ARA criteria (11). The mean age was 51 years, and the mean duration of disease nine years. Rheumatoid factor was positive in nine of the patients when tested by the Waaler-Rose technique. The scleroderma patients had a mean age of 55 years and a mean disease duration of six years. The patients with the other diagnoses were grouped together and called seronegative spondarthritis. Their mean age was 41 years; the mean duration of the disease 10 years.

The patients with scleroderma were, at the time of this study, treated with cyclofenil, on average 400 mg/day. All the other patients had been treated with various nonsteroidal antiinflammatory drugs, which were ceased three to four days prior to blood sampling. None of the patients had been treated with penicillamine or corticosteroids, and all other disease-modifying drugs were withdrawn at least three months prior to the study. None of the patients had any laboratory signs of liver disease. None suffered from diabetes mellitus, infection, or malignancy. There were no significant differences in energy, protein, fat, or carbohydrate intake during the study, and the food selection patterns remained the same during the observation period. None of the patients was obese. Laboratory data reflecting the degree of the inflammatory activity are presented in Table 1.

The controls consisted of healthy individuals (nine males and six females, mean age 43 years) from the Uppsala region without any kind of therapy, eating a standard Swedish diet.

Cellular and Plasma Zinc. Ten milliliters of venous blood was drawn into venoject tubes (Terumo, Tokyo, Japan) with 0.1 ml 0.38 M K$_2$EDTA as anticoagulant. Erythrocytes, granulocytes, and platelets were immediately isolated from the blood sample as previously described (12). After washing in sterile phosphate-buffered saline (PBS), pH 7.3, (SBL, Stockholm, Sweden), the different cell types were resuspended in 1 ml 0.32 M sucrose (Analar/BDH, London, England) free from Zn, Ca, and Fe. Then 0.5-μl aliquots (equivalent to approximately $1 \times 10^5$ erythrocytes, $0.5 \times 10^4$ granulocytes, and $0.5 \times 10^6$ platelets, respectively) were put onto clean Formvar films (Formvar 1595 E; Merck, Darmstadt, West Germany). Following freezing to $-80^\circ$C, the cells were freeze-dried and stored in desiccators at $+4^\circ$C.

From the blood cell preparations, ten cells of each type were selected under the light microscope prior to microprobe analysis. A technical account of the analytical instrument has been pub-