

The Relationship between Carbonic Anhydrases and Zinc Concentration of Erythrocytes in Patients under Chronic Hemodialysis*

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ABSTRACT

The levels of carbonic anhydrase I and II isozymes in human erythrocytes were determined using the single radial immunodiffusion method in 53 patients (29 males, 24 females) under chronic hemodialysis, and zinc concentration in erythrocytes were also determined.

Both carbonic anhydrase I and II levels and zinc concentration in erythrocytes were observed to be significantly increased in chronic hemodialysis patients as compared against healthy adults (carbonic anhydrase I: $p < 0.001$, carbonic anhydrase II: $p < 0.001$, zinc: $p < 0.001$).

In chronic hemodialysis patients, a significant positive correlation was found between both carbonic anhydrase I and II and zinc concentration in erythrocytes (carbonic anhydrase I and zinc: $p < 0.01$, carbonic anhydrase II and zinc: $p < 0.02$).

These results suggest that increase of carbonic anhydrases is a cause for increase of zinc concentration in erythrocytes of chronic hemodialysis patients.

INTRODUCTION

Three isozymes have been demonstrated in human carbonic anhydrase, EC 4.2.1.1 (hereafter abbreviated as CA), namely, CA I (B), CA II (C)¹⁷⁾ and CA III¹⁴⁾. Although it has not been completely elucidated yet how these isozymes are distributed in the tissues, it is considered that CA I exists mostly in erythrocytes, gastric mucosa and kidney, CA II in erythrocytes and a wide variety of tissues, and CA III in skeletal muscles. Further, the CA levels are known to vary with various diseases; for example, Mondrup et al.¹¹⁾ and Yamakido et al.²⁰⁾ have reported that the CA levels increase in chronic renal failure.

On the other hand, it has been reported that the zinc concentration in erythrocytes increases

in chronic renal failure^{9,10,15)}, but the mechanism of this has not yet been clarified. As CA is a metallo enzyme with a molecular weight of about 30,000 having one atom of zinc in one molecule, increase of zinc concentration in erythrocytes in chronic renal failure is presumed to be related to increase of the CA levels. As regards the zinc concentration and CA levels in erythrocytes, however, although Vallee et al.¹⁸⁾ have reported a correlation of CA activity to the zinc concentration, no report has yet been made of a study concerning its relationship to the CA levels. Accordingly, the authors attempted to elucidate the mechanism of increase of the zinc concentration in erythrocytes in chronic renal failure by determining the CA I and II levels and zinc concentration in the erythrocytes of chronic hemodialysis patients

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and studying the correlation between them.

SUBJECTS AND METHODS

1) *Subjects*

The study subjects are 53 chronic hemodialysis patients (29 males and 24 females), whose mean age is 46.4 ± 14.5 (mean \pm standard deviation), and whose mean years of hemodialysis is 3.3 ± 2.2 . Their controls are 37 healthy adults (13 males and 24 females) whose mean age is 37.2 ± 12.1 years.

2) *Determination method of CA in erythrocytes*

(1) Purification of CA

CA in human erythrocytes was purified according to the method of Osborne and Tashian¹²⁾. First, Prontosil was synthesized from *p*-aminobenzenesulfamido and *m*-phenylenediamine by the the method of Sakai et al.¹⁰⁾ Next, the Prontosil was coupled with CM Sephadex C-50 to produce affinity columns. Then, CA I and CA II were separated from the hemolysate and purified using DEAE Sephadex A-50 columns. The concentrations of E_{1cm}^{1%} 16.3 and 18.7 were obtained for CA I and CA II, respectively¹⁾.

(2) Preparation of CA specific antisera

One mg each of purified CA I and CA II

was immunized together with Freund's complete adjuvant on foot pads of rabbits. After 3 more immunizations made at intervals of one week, antisera were prepared.

(3) Determination of CA levels

CA levels were determined by the SIRD method. That is, mixing anti-CA I and anti-CA II antisera with 1.2% agar, agar plates were prepared. Then, measuring lines were determined from the diameters of the precipitation rings indicated by standard solutions of three different concentrations, and from these lines, the CA I and CA II levels in the hemolysate specimens were obtained. The hemoglobin concentration was measured by the cyanmethemoglobin method, and the CA levels were expressed in terms of mg/g hemoglobin.

3) *Determination method of zinc in erythrocytes*

Quantitation of zinc in erythrocytes was made using the Nippon JARRELASH atomic absorption spectrophotometer, on erythrocytes collected with a syringe ascertained to be uncontaminated with zinc, washed three times in physiological saline, and diluted 20 times in deionized water. As standard zinc solution, that for use in atomic absorption spectrophotometry (1,000 ppm, Wako Jun-yaku Co.) diluted in

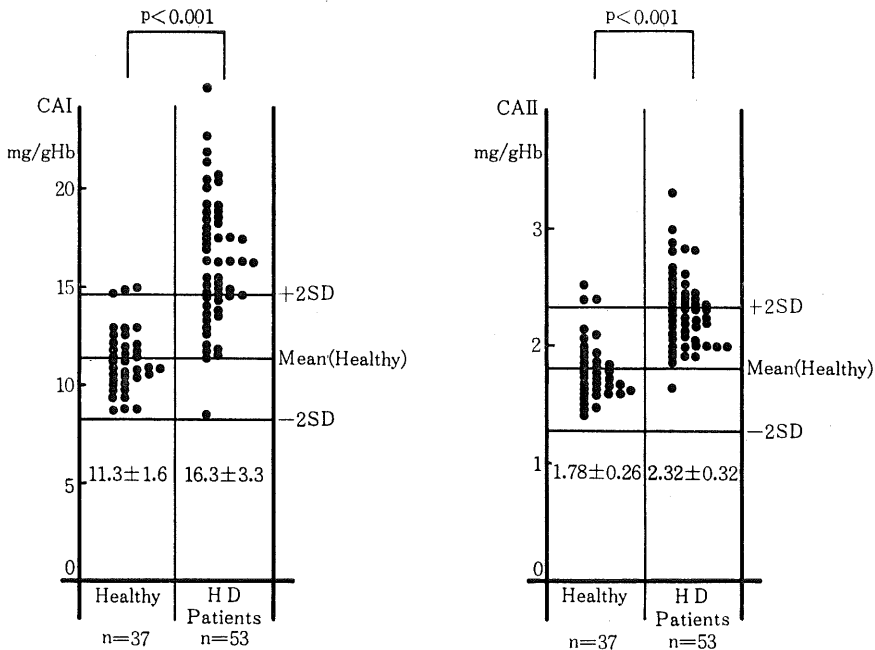


Fig. 1. Carbonic anhydrase I and II levels in patients under chronic hemodialysis

deionized water was used.

RESULTS

1) *CA I and CA II levels in erythrocytes (Fig. 1)*

The CA I level in 37 cases in the healthy group was 11.3 ± 1.6 mg/gHb (mean \pm standard

deviation), and the CA II level 1.78 ± 0.26 mg/gHb. On the other hand, the CA I level in chronic hemodialysis patients was 16.3 ± 3.3 mg/gHb, showing a significant increase ($p < 0.001$) compared with the healthy group. Similarly, the CA II level in chronic hemodialysis patients was 2.32 ± 0.32 mg/gHb, also showing

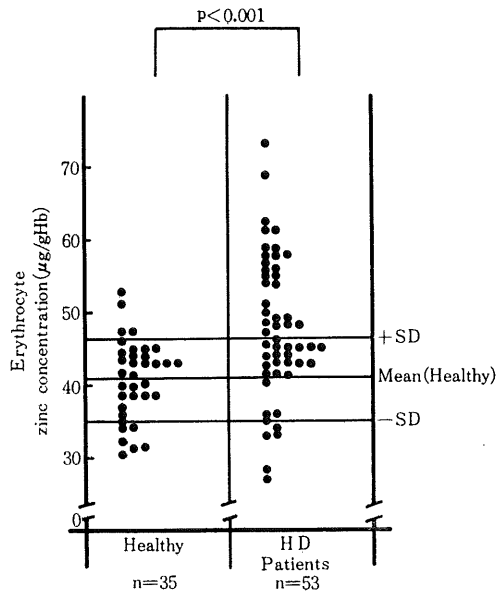


Fig. 2. Erythrocyte zinc concentration in patients under chronic hemodialysis

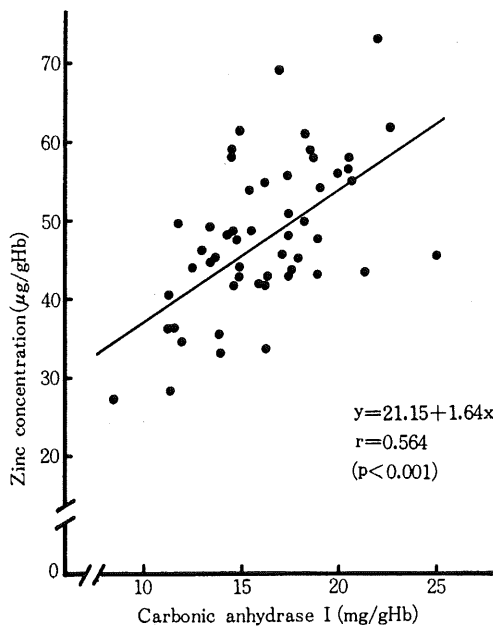


Fig. 3. Correlation between carbonic anhydrase I levels and zinc concentration

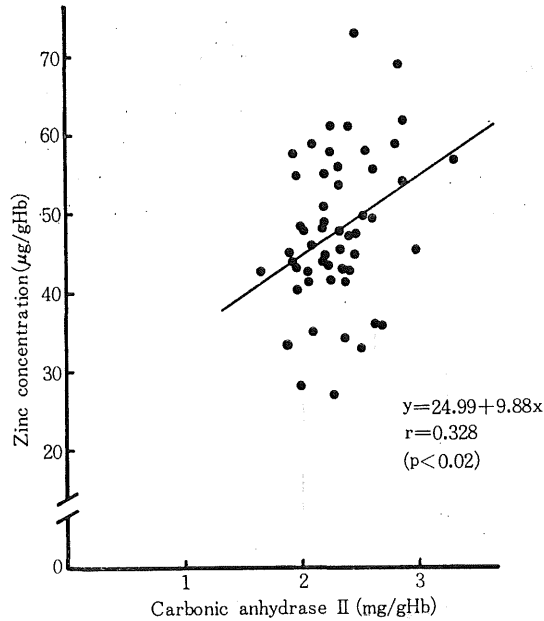


Fig. 4. Correlation between carbonic anhydrase II levels and zinc concentration

a significant increase ($p<0.001$) compared with the healthy group.

2) *Zinc concentration in erythrocytes (Fig. 2)*

The zinc concentration in erythrocytes in 35 healthy adults was $40.8 \pm 5.5 \mu\text{g/gHb}$, while that in chronic hemodialysis patients was $47.9 \pm 0.7 \mu\text{g/gHb}$, showing a significant increase ($p<0.001$) compared with the healthy group.

3) *Relationship between CA I and CA II levels in erythrocytes and zinc concentration in erythrocytes (Fig. 3 and Fig. 4)*

The zinc concentration in erythrocytes increased with increase of the CA I levels in erythrocytes, showing a significant positive correlation between the CA I levels and the zinc concentration ($p<0.001$).

Significant positive correlation was also observed between the CA II levels and the zinc concentration.

DISCUSSION

It has been reported that the CA levels in human erythrocytes is increased in hypothyroidism^{8, 8, 13, 19}, chronic obstructive lung disease^{3, 5} and iron deficiency anemia^{4, 7}, while it is decreased in hyperthyroidism^{8, 8, 13, 19} and polycythemia vera⁹. Few reports have been made concerning the CA levels in erythrocytes in renal failure, but Mondrup¹¹ and Yamakido et al.²⁰, have reported increased CA levels. It is

considered that increased CA I and CA II levels in chronic renal failure compensate for acid-base balance abnormality in the renal tubulus and also for the possible decreased ability to transport CO_2 from the tissues to the lungs in these patients who present anemia.

On the other hand, Vallee et al.¹⁸, Condon et al.², and Mahajan et al.^{9, 10} have reported with regard to the movement of zinc in chronic renal failure, increased zinc concentration in erythrocytes and decreased zinc concentration in blood plasma. As CA contains zinc, movements of CA and zinc are of great interest. Furthermore, Vallee et al.¹⁸ have found on making determinations of the zinc concentration and CA activity in erythrocytes at the same time that the two are well correlated in anemia.

Since the isozymes CA I and CA II are present in human erythrocytes and can be determined immunologically, the relationship of these isozymes and zinc will become more explicit by studying the relationship between the levels of these isozymes and zinc concentration. As observed in the results obtained by the authors in the present study, the levels of both CA I and CA II showed significantly high values in the group of chronic hemodialysis patients, and the zinc concentration in erythrocytes likewise showed significantly high values. Further, the zinc concentration and CA I and CA II levels

in erythrocytes showed a significant positive correlation, and it can be construed from this that not only CA I, but CA II also is involved in the increase of the zinc concentration in erythrocytes.

The zinc concentration in blood plasma, leukocytes and hair are decreased in patients of chronic renal failure compared with normal subjects¹⁰. These zinc metabolic abnormalities do not improve with hemodialysis and peritoneal dialysis, and their mechanisms have not been thoroughly clarified yet. The results of the authors' present study will be very helpful in future studies.

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