Human Erythrocyte Carbonic Anhydrase B and C in Chronic Obstructive Lung Disease

TAKAHITO KONDO, NAOYUKI TANIGUCHI,* YOSHIKAZU KAWAKAMI, HAJIME IDE, KAZUO SAITO* and MAKOTO MURAO

The First Department of Internal Medicine and Department of Hygiene and Preventive Medicine, Hokkaido University School of Medicine, Sapporo*

KONDO, T., TANIGUCHI, N., KAWAKAMI, Y., IDE, H., SAITO, K. and MURAO, M. *Human Erythrocyte Carbonic Anhydrase B and C in Chronic Obstructive Lung Disease*. Tohoku J. exp. Med., 1975, 117 (4), 351-356 — The levels of carbonic anhydrase B and C isozymes in human red cells were determined using a quantitative immunological technique in patients with chronic obstructive lung disease. A significant increase in the level of carbonic anhydrase B was observed in these patients, while the level of carbonic anhydrase C did not change substantially. Positive correlations were found between the level of carbonic anhydrase B and arterial CO_2 tension and plasma HCO_3^- concentration. A negative correlation was observed between the levels of carbonic anhydrase B and blood pH. These findings suggest that the synthesis or degradation of carbonic anhydrase B isozyme is affected by arterial CO_2 tension or plasma HCO_3^- concentration. The clinical significance was also discussed in relation to these isozyme levels in red cell. — carbonic anhydrase B and C isozymes; chronic obstructive lung disease; hypercapnia

Human carbonic anhydrase (E C 4.2.1.1) has two major types of isozymes designated as B type (CA-B) and C type (CA-C). Immunologically these two types of isozymes do not show any cross reaction with each other (Funakoshi and Deutsch 1970) and were genetically controlled by different loci of the gene (Tashian 1969; Deutsch et al. 1972b). As a catalyzer for the reversible hydration of CO_2 , the enzyme plays an important role in the transport of CO_2 and acid base equilibrium (Dubois 1969; Lindskog et al. 1971; Maren 1971). The levels of CA-B and CA-C are changed under some pathological or physiological conditions as reported previously (Funakoshi and Deutsch 1971; Matsuo 1972; Mochizuki 1972; Mondrup and Anker 1974; Sell and Petering 1974; Taniguchi et al. 1975; Kondo et al. 1975). Chronic obstructive lung disease (C.O.L.D.) is defined as a disease with chronic obstruction to airflow in the lungs. The common causes are emphysema, chronic bronchitis, and certain forms of asthma (Thurbeck et al. 1970). Patients with C.O.L.D. have a reduced capacity to eliminate CO_2 from the alveoli and hence tend to develop chronic hypercapnia. It is of interest to know the changes in levels of carbonic anhydrase isozymes under these conditions. This

paper describes the levels of erythrocyte CA-B and CA-C using immunological technique in the patients with C.O.L.D. in order to examine whether or not the changes in arterial CO_2 tension (Paco_2) or plasma HCO_3^- concentration affect the levels of erythrocyte CA-B or CA-C.

MATERIALS AND METHODS

Subjects. Thirty six blood samples obtained from eighteen patients diagnosed as C.O.L.D. in various clinical stages, admitted to the Hokkaido University Hospital, were used for this study. C.O.L.D. patients accompanied with kidney or blood disorders were excluded. Sixteen patients were male and two were female, with an average age of 60 years. They had Paco_2 levels of 47.0 ± 1.58 mmHg (mean $\pm$ s.e.) ranging from 35 mmHg to 76.5 mmHg, pH values of 7.364 ± 0.01 (mean $\pm$ s.e.) ranging from 7.290 to 7.412, and HCO_3^- concentrations of 26.17 ± 0.80 mEq/liter (mean $\pm$ s.e.) ranging from 20.0 mEq/liter to 41.5 mEq/liter. The levels of CA-B and CA-C in normal adults were reported elsewhere (Taniguchi et al. 1974; Kondo et al. 1975). These values were employed in the present work as control. Determination of Paco_2 and blood pH was measured by a blood gas microanalyzer (Radiometer, BMS-MK, 2, Copenhagen). Plasma HCO_3^- concentration was determined using an acid-base alignment nomogram according to Siggaard-Andersen (Siggaard-Andersen 1960).

Purification of carbonic anhydrase B and C types. Human red cell carbonic anhydrase isozymes were purified with a slightly modified method (Taniguchi et al. 1974) used for horse enzymes originally by Deutsch et al. (1972a).

Specific antisera. Specific antisera against carbonic anhydrase isozymes were prepared by subcutaneous injection of purified enzymes with Freund's complete adjuvant in draft horses as described previously (Taniguchi et al. 1975). Antisera thus obtained showed a single precipitin line to each isozyme.

Preparation of hemolysates. One ml of arterial blood collected in heparinized tubes was used as specimens. Whole blood was centrifuged to remove the plasma, and packed blood cells were washed twice with ten volumes of isotonic saline by subsequent centrifugation. By repeated freezing at -80°C and thawing, packed blood cells were hemolyzed. Stroma was removed from the hemolysates by centrifugation at $10,000 \times g$ for 30 min. Hemoglobin contents of hemolysates were measured by the cyanmethemoglobin method (Van Kampen and Zijlstra 1961). The hemoglobin content of hemolysates was adjusted to approximately 5 g/100 ml with isotonic saline before the immunological assay.

Immunological measurement. CA-B and CA-C isozymes were assayed according to the method described previously (Dubois 1969). Antigen concentration was determined spectrophotometrically at 280 nm using $E_{1\text{cm}}^{1\%}$ at 280 nm = 16.3 for CA-B and 18.7 for CA-C, respectively (Nyman and Lindskog 1974). The levels of CA-B and CA-C were expressed as mg enzyme per g hemoglobin.

RESULTS

Carbonic anhydrase B and C levels in patients with chronic obstructive lung disease and normal subjects

Table 1 shows the mean and standard errors of the levels of CA-B and CA-C in the red cells of patients with C.O.L.D. and these in normal subjects. The levels of CA-B in patients with C.O.L.D. were significantly higher than those in

TABLE 1. *Levels of human carbonic anhydrase*

Subjects	Number of cases	CA-B (mg/g-Hb s.e.)	CA-C
Normal male (Taniguchi et al. 1974; Kondo et al. 1975)	94	13.08±0.42	1.70±0.03
Normal female (Taniguchi et al. 1974; Kondo et al. 1975)	40	14.21±0.86	1.74±0.04
C.O.L.D.	36	17.90±0.58	1.58±0.09

Values are expressed as mean±s.e. C.O.L.D., chronic obstructive lung disease.

normal subjects ($p<0.001$), while no substantial differences were observed in the levels of CA-C.

Relations between the levels of carbonic anhydrases and P_{aCO_2} , plasma HCO_3^- concentration and blood pH

The levels of CA-B in red cells were positively correlated to P_{aCO_2} and plasma HCO_3^- concentration. As shown in Figs 1. and 2. the correlation coefficient showed r values of 0.703 ($p<0.01$) and 0.604 ($p<0.01$), respectively. No correlations were seen between the levels of CA-C and the P_{aCO_2} or plasma HCO_3^- concentration. As shown in Fig. 3, CA-B level increases as blood pH decreases ($r=0.351$, $p<0.05$). P_{aCO_2} levels of 45 mmHg and plasma HCO_3^- levels of 25 mEq/liter are considered to be the upper limit of normal ranges. According to Fig. 1, patients with P_{aCO_2} of 45 mmHg or higher have higher levels CA-B than those of normal subjects. As shown in Fig. 2, the levels of CA-B were elevated when HCO_3^- concentration increased over 25 mEq/liter.

Fig. 1. Correlation between P_{aCO_2} and CA-B in patients with C.O.L.D.

Fig. 2. Correlation between HCO_3^- and CA-B in patients with C.O.L.D.

Fig. 3. Correlation between blood pH and CA-B in patients with C.O.L.D.

DISCUSSION

Patients with C.O.L.D. were shown to have relatively higher levels of carbonic anhydrase B in red cells than those of normal healthy adults. The results obtained also suggested that the changes in Paco_2 of plasma, HCO_3^- concentrations and blood pH are well correlated to the changes in CA-B levels in red cells. Patients with normal ranges of Paco_2 and plasma HCO_3^- concentrations have a normal value of CA-B in red cells. Patients with the values over normal ranges, however, showed increased levels of CA-B. It is of interest to ascertain whether high levels of carbonic anhydrase have any significant influence on the CO_2 exchange in the lung capillaries of C.O.L.D. Roughton (1964) emphasized the importance of carbonic

anhydrase in the physiological transport of CO_2 and described the possible influence of the activity of carbonic anhydrase on CO_2 exchange in the lung tissues. Funakoshi and Deutsch (1971) reported that patients with emphysema had high levels of erythrocyte CA-B, and Lie-Injo et al. (1970) reported that patients with chronic lung disease had a high activity of this enzyme. The present results are in good agreement with these previous studies.

Hypoventilation and ventilation/perfusion abnormalities in C.O.L.D. results in the CO_2 retention and in severe cases give rise to hypercapnia or respiratory acidosis. In C.O.L.D., an adaptive increase in CA-B levels in red cells may well occur in order to meet with an adequate CO_2 exchange in lungs and tissues. The regulatory mechanism of the levels of carbonic anhydrase in red cells, however, is not well known. As to whether CO_2 tension in the alveoli affects primarily the carbonic anhydrase levels in red cells is also not well understood.

Our previous report indicated that the esterase activities of CA-B increased proportionally as the increase of CA-B protein in patients with C.O.L.D., and so "true" specific activity of CA-B enzyme was within normal ranges (Kondo et al. 1975). These facts also suggest that an adaptive increase in CA-B protein may occur and CA-B enzyme protein was revealed to be functionally active in these patients. These increases in CA-B levels might depend on the increase in CA-B protein synthesis or the decrease in the degradation of this enzyme protein. We are at present continuing work along this line to clarify this point.

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