Tr. J. of Medical Sciences
28 (1998) 407-410

T. Abdülkadir ÇOBAN[1]
Barbaros NALBANTOĞLU[2]
M. Yaşar ÇİL[3]
Hasan ÖZDEMİR[2]
Ö. İrfan KÜFREVİOĞLU[2]

Investigation of the Inhibition Effects of Some Antibiotics on Human Erythrocyte Carbonic Anhydrase Isozymes

Received: September 10, 1996

[1]Department of Chemistry Education, Erzincan Education Faculty, Atatürk University, Erzurum-Turkey
[2]Department of Biochemistry, Faculty of Science & Arts, Atatürk University, Erzurum-Turkey
[3]Department of Biochemistry, Faculty of Science & Arts, Kahramanmaraş Sütçü İmam University, Kahramanmaraş-Turkey

Abstract: In this study, the in vitro effects of some antibiotics on carbonic anhydrase (CA) isozymes have been investigated. Human erythrocyte CA-I and CA-II isozymes were separately purified by affinity chromatography. Inhibition effects of four different antibiotics on CA isozymes were determined by using the CO_2-Hydratase method. I_{50} values of antibiotics were obtained by drawing % Activity [Actibiotic] graphs. The obtained I_{50} values of cefazolin sodium, cephradine, sulbactam/cefoperazone and chloramphenicol sodium succinate were 9, 16, 19 and 48 mM on CA-I and 6, 10, 15 and 17 mM on CA-II, respectively. Antibiotics dosages should be taken into consideration because of the inhibition effect on CA which is pH regulatory enzyme in body.

Key Words: Carbonic anhydrase isozymes, antibiotics, inhibition.

Introduction

Carbonic anhydrase (CA) has been a well characterized pH regulatory enzyme in most tissues including erythrocytes (1). CA catalyzes the reversible hydration of CO_2 to HCO_3^- and H^+. To date six isozymes have been described in mammals. CA-I is found together with CA-II in erythrocytes; CA -III is the most abundant soluble protein in skeletal muscle; CA-IV, a membrane-bound form, has been isolated from the brush-border and basolateral membranes and microsomes of tubular cells of human kidneys and human lung; CA V is found in mitochondria of certain tissues and CA VI is secreted into the saliva (2). It has been reported that activity levels of CA isozymes in human erythrocytes vary considerably under certain pathological and physiological conditions. Changes in CA activity have been associated with metabolic diseases like diabetes mellitus and hypertension (1, 3). It has been reported that inhibition of CA was found to impair proton secretion into the proximal tubule lumen and thereby decreased bicarbonate reabsorbtion. At the same time, inhibition of CA also decreased the rate of acidification of urine, producing alkaline urine and eventually metabolic acidosis (4). Therefore, when parenteral sulfonamides (i.e., acetazolamide and methazolamide, carbonic anhydrase inhibitors) are used in therapies, useful inhibitor dosages have been suggested.

Many antibiotics have been used in therapies. There are few literature reports related with changing of enzyme activities. It has been reported that some increasing or decreasing were found on human liver enzyme activity levels such as aspartate aminotransferase (AST;SGOT), alanine aminotransferase (ALT;SGPT) and alkaline fosfatase (5-9). Since the effects of some antibiotics have not been analyzed on CA isozymes which are contained at the highest molar amounts in erythrocytes, in the present study, the in vitro effects of cefazolin sodium, cephradine, sulbactam/cefoperazone and chloramphenicol sodium succinate on CA-I and CA-II purified from human erythrocytes were investigated. By using the obtained I_{50} values (inhibitor-enzyme binding costants), undesirable side-effects can be diminished on CA activity and body metabolism in therapy.

Materials and Metohds

Materials: Sepharose 4B and Sephadex G-25 were obtained from Sigma Chem. Co., p-aminobenzenesulfonamide and L-tyrosine were from E.Merck, and cefazolin sodium, cephradine, sulbactam/cefoperazone and chloramphenicol sodium succinate were from Pharmacy. The blood samples with ACD (acid-citrate-dextrose) were obtained from healthy persons.

Purification of Carbonic Anhydrase Isozymes from

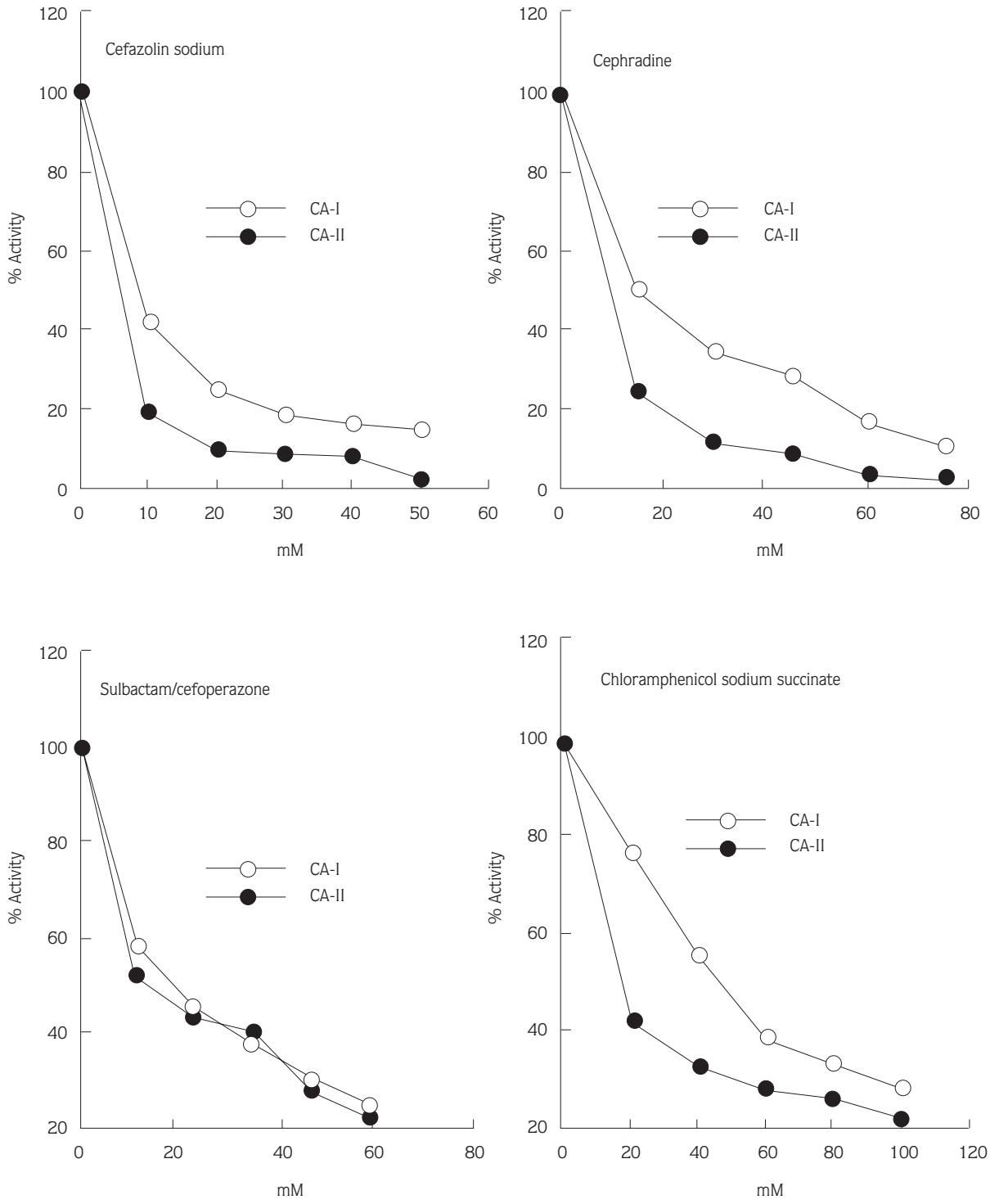

Figure 1. % Activity-[Antibiotic] graphs for CA-I and CA-II in the presence of four different antibiotics.

Human Erythrocytes: Erythrocytes were purified from human blood obtained from the Blood Center of the Research Hospital at Atatürk University. The blood samples were centrifuged at 1500 rpm for 20 min. and the plasma and buffy coat were removed. After the packed red cells were washed with NaCl (0.9 %) two

Table 1. I_{50} values obtained from Regression analysis graphs for CA-I and CA-II in the presence of four different antibiotics.

	I_{50} mM	
ANTIBIOTIC	CA-I	CA-II
Cefazolin sodium	9	6
Cephradine	16	10
Sulbactam/cefoperazone	19	15
Chloramphenicol sodium succinate	48	17

times, the erythrocytes were hemolyzed with cold water. The ghost and intact cells were removed by centrifugation at 4˚, 20.000 rpm for 30 min. The pH of the hemolysate was adjusted to 8.5 with solid Tris. The hemolysate was applied to the prepared Sepharose-4B-L-tyrosine-sulfanylamide affinity column (10) equilibrated with 25 mM Tris-HCl/0.1 M Na_2SO_4 (pH 8.5). The affinity gel was washed with 25 mM Tris-HCl/22 mM Na_2SO_4 (pH 8.5). The human carbonic anhydrase (CA-I and CA-II) isozymes were eluted with 1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M $NaCH_3COO$/0.5 M $NaClO_4$ (pH 5.6), respectively (10).

Determination of Protein Content and Carbonic Anhydrase Activity: For protein content estimation the method of Coomassie brilliant blue (11) was used with bovine serum albumin as a standart. The absorbance at 280 nm was used to monitor protein in the column effluents. Carbonic anhydrase activity was assayed by following the hydration of CO_2 according to the method of Wilbur and Anderson (12). CO_2 - Hydratase activity as enzyme unit (EU) was calculated by the equation (t_0-t_c/t_c) where t_0 and t_c are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively. The purification was controlled with SDS-polyacrylamide gel electrophoresis (13).

Inhibition Studies of Antibiotics: Antibiotics such as cefazolin sodium, cephradine, sulbactam/cefoperazone and chloramphenicol sodium succinate were used. Five different volumes (0.1, 0.2, 0.3, 0.4 and 0.5 ml) of antibiotics at constant concentration were added to 0.1 ml enzyme solution in order to incubate them form 3 min.. Carbonic anhydrase activities with antibiotics were assayed by following the hydration of CO_2 (12). % Activity values of CA-I and CA-II for five different concentration of each antibiotic were drawn by using Regression analysis graphs. Carbonic anhydrase activity without antibiotic was accepted as 100 % Activity. The inhibitor concentrations causing up to 50 % inhibition (I_{50}) were determined from the graphs.

Results and Discussion

Many chemicals at relatively low dosages effect the metabolism of biota by altering normal enzyme activity (14). With some of these interactions there is a high reactivity involving a high degree of inhibition of a specific enzyme that accounts for the effect on the whole animal or plant (15). It has been reported that antibiotics are used as a drug and have some side-effects which cause increasing or decreasing on human liver enzyme activity levels such as aspartate aminotransferase (AST;SGOT), alanine aminotransferase (ALT;SGPT) and alkaline fosfatase (5-9). Although antibiotics are used in therapies, there is no report related with CA isozyme activities. Indeed, CA isozymes are important enzymes for body metabolism because it regulates pH in most tissues.

Therefore, in the present study, investigation of in vitro effects of antibiotics on human erythrocyte CA-I and CA-II was proposed. In order to make this study, CA-I and CA-II were separately purified from human erythrocytes by Sepharose-4B-L-tyrosine-sulfanylamide affinity chromatography. Cefazolin sodium, cephradine, sulbactam/cefoperazone and chloramphenicol sodium succinate as antibiotics were chosen for investigation of antibiotic effects. Some researchers use I_{50} value causing 50 % inhibition of enzyme activity to show inhibition effect. Therefore, in this study, determination of I_{50} values of antibiotics was planed.

I_{50} values were determined by drawing % Activity-[Antibiotic] graphs on both CA-I and CA-II by using five different concentrations for eacch antibiotic as shown in Figure 1. The obtained I_{50} values of cefazolin sodium, cephradine, sulbactam/cefoperazone and chloramphenicol sodium succinate were 9, 16, 19 and 48 mM on CA-I and 6, 10, 15 and 17 mM on CA-II, respectively, shown in Table 1. These results show that these four antibiotics inhibite CA-I and CA-II at low mM concentrations and have lower I_{50} values on CA-II than CA-I. Since cefazolin sodium has the lowest I_{50} values on both CA-I and CA-II, it has most inhibition effect among the used antibiotics.

It is generally recognized that CA controls the bulk of carbon dioxide exchange between the blood and tissues as well as the regulation of proton and other ion movements between cells and extracellular fluids. All of the CA isozymes are also deeply involved with a great number of secretory activities including fluid movements (16). Since CA is very important enzyme for body, the inhibition effects of these antibiotics should be considered for not

only erythrocyte CA-I and CA-II but also all CA isozymes. For example, in two recent studies, liver total CA (I+II+III+V) activity was diminished in streptozotocin induced diabetic rat (17, 18). They have also demonstrated the gluconeogenesis and ureagenesis with an increase in isozyme CA-V activity in hepatocytes (17, 19). They have shown pH disequilibrium in rat liver that could be explained by the changes in CA activity. Also, many of the side-effects observed are probably due to inhibition of CA isozymes (2). For example, the respiratory acidosis is probably responsible for some of the side reactions observed during acetazolamide (inhibitor of CA) therapy: i.e. fatigue, headache, taste sensations and for the distress in the "chronic lunger" (2). When the earlier acetazolamide inhibition studies and the present in vitro study are considered together, it is

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