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Effects of Some Antibiotics on Enzyme Activities of Carbonic Anhydrase from Erythrocytes *in Vivo* and *in Vitro*

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Abstract

In this study, firstly, the effects of eight antibiotics namely, chlarithromycin, chloramphenicol, rifamycin SV, clindamycin phosphate, ciprofloxacin, gentamycin sulfate, sodium ampicillin and cefazolin sodium, on human carbonic anhydrase (hCA) (EC 4.2.1.1.) isozymes have been investigated *in vitro.* Human erythrocytes CA-I and CA-II isozymes were separately purified by affinity chromatography. Inhibition effects of eight different antibiotics on hCA isozymes were determined using the CO_2 hydratase method by plotting activity % *vs* [medical drug]. IC_{50} values of the antibiotics exhibiting inhibition effects were found by means of these graphs. It was observed that each antibiotics showed an inhibition effect on hCA-I and hCA-II hydratase activity. Sodium ampicillin showed greatest inhibition effect on hCA-I and hCA-II activity from *in vitro* studies. In order to determine the inhibition statue of these drugs on a living system, the effects of same antibiotics on CA enzyme activity of mouse erythrocytes was investigated *in vivo*. Ciprofloxacin (3.2 mg/kg), chlarithromycin (7.14 mg/kg), rifamycin SV (3.56 mg/kg) lead to the significant increase in mouse erthrocytes CA (mCA) after 4 h drug administration. As well gentamycin sulfate (3.2 mg/kg) and sodium ampicillin (17.12 mg/kg) after 2 h and clindamycin phosphate (2.143 mg/kg) after 2 h and 6 h drug administration, lead to increase in mCA. Chloramphenicol (12.5 mg/kg) did not exhibit any effect for the mCA, *in vivo.* However, CA activity after sodium ampicillin and cefazolin sodium administration showed statistically significant decrease at 4 h of drug administration *in vivo* (p: 0.014 and 0.016, respectively).

Key Words

Carbonic anhydrase,
Erythrocyte,
Antibiotics,
in vivo,
in vitro

Abbreviations

hCA: human carbonic anhydrase; CA-I: carbonic anhydrase one; CA-II: carbonic anhydrase two; mCA: mouse erythrocytes

INTRODUCTION

The carbonic anhydrases (CAs, EC 4.2.1.1) are widespread metalloenzymes in all living organisms including humans [1-6]. Indeed, by catalyzing a simple but fundamental reaction, the reversible hydration of CO_2 to bicarbonate and a proton, these metalloenzymes are involved in a multitude of physiological and pathological processes, and their inhibition leads to responses that may be exploited therapeutically. Besides 16 isoforms have been described up to now, [7-9] of which at least CA II, IV,

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VA, VB, VII, IX, XII, XIII, and XIV constitute valid targets for the development of novel antiglaucoma, antitumor, antiobesity or anticonvulsant drugs [10-15].

It has been reported that the activity levels of CA isozymes in human (HCA-I and HCA-II) erythrocytes vary considerable under certain pathological and physiological conditions. Changes in CA activity have been associated with metabolic disease such as diabetes mellitus and hypertension [16,17]. It has been reported that the inhibition of CA was found to impair proton secretion into the proximal tubule lumen and thereby decreased bicarbonate reabsorption. At the same time, inhibition of CA also decreased the rate of acidification of urine, producing alkaline urine and eventually metabolic acidosis [18].

CA enzymes purified from various organisms have been shown to be inhibited by various compounds. Sulfonamides, like acetozolamide and heavy metals are considered as the strongest CA inhibitors [19]. In addition, some *in vitro* and *in vivo* studies showed that some antibiotics including streptomycin sulfate, sodium dipyrone, magnesium sulfate, sodium ceftizoxime, sodium cefuroxime, sodium cefoperazone, stroptomycine sulfate and methylmycine sulfate, some chemicals and, finally, some pesticides also inhibit CA enzyme activity to a wide range of degrees [20,21].

Antibiotics are drugs derived wholly or partially from certain microorganisms and are used to treat bacterial or fungal infections. They are ineffective against viruses which either kill microorganisms or stop them from reproducing [22]. The great number of diverse antibiotics currently available can be classified in different ways, e.g., by their chemical structure, their microbial origin, or their mode of action. They are also frequently designated by their effective range.

Macrolid, amphenicol, aminoglicoside, and penicillin derivative antibiotics the most widely used broad-spectrum antibiotics, are effective against both Gram-positive and Gram-negative bacteria [23]. Beta-lactams are compounds with a beta-lactam ring in their structure. They comprise principally the penicillins and the cephalosporins, but also they include clavulanic acid. Theaminoglicoside are products of actinomycetes (soil bacteria) or semi-synthetic derivatives of the natural products [24]. The beta-lactam antibiotics bind to enzymes and inhibit them needed for the synthesis of the peptidoglycan wall. While they have little effect on resting bacteria, they are lethal to dividing bacteria as defective walls cannot protect the organism form bursting in hypotonic surroundings.

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 enzyme activity levels were found on human liver such as aspartate aminotransferase (AST; SGOT), alanine amino-transferase (ALT; SGPT) and alkaline fosfatase [25-29]. Since the effects of some antibiotics have not been analyzed on CA isozymes which are contained at the highest molar amounts in erythrocytes, *in vivo* and *in vitro*. The interaction between human erythrocyte lysates and antibiotics was studied, and the effect of intracellular components on the activity and binding of the drugs was determined by Kornguth *et al.* [30]. In the studies using pure preparations of carbonic anhydrase revealed that the CA isozyme is the major binder of the tetracyclines and zinc did not inhibit enzymatic activity of carbonic anhydrase.

Another study about carbonic anhydrase in bone resorption induced by 1,25 dihydroxyvitamin D3 *in vitro*. The most significant finding was that 1, 25(OH) 2D3-induced calcium release was accompanied by a significant increase in the carbonic anhydrase

activity of bone at both 48 h (treated/control ratio = 2.05) and 96 h (treated/control ratio = 2.59) [31]. Cefazolin sodium, cephradine, and sulbactam/ cefoperazone and chloramphenicol sodium succinate antibiotics were studied by Coban et. al. and in this study the IC_{50} values were indicated as of 9, 16, 19 and 48 mM on hCA I and 6, 10, 15 and 17 mM on HCA II, respectively [32]. But there is no report about carbonic anhydrase isozymes HCA-I and HCA-II with the inhibition of chlarithromycin, chloramphenicol, rifamycin SV, clindamycin phosphate, ciprofloxacin, gentamycin sulfate, sodium ampicillin, and cefazolin sodium (Figure 1). In the present study, the *in vitro* effects of these antibiotics on hCA isozymes purified from human erythrocytes and the *in vivo* effect on mCA enzyme from mice erythrocytes were investigated.

MATERIALS AND METHODS

Sepharose 4B, L-tyrosine, sulfanylamide, protein assay reagents, phenol red and chemicals for electrophoresis were obtained from Sigma Chem. Co. (Milano, Italy). All other chemicals were analytical grade. Medical drugs were provided by local pharmacy.

Purification of carbonic anhydrase isozymes from human erythrocytes by affinity chromatography.

Erythrocytes were purified from fresh human blood obtained from the State Hospital in Balikesir. The blood samples were centrifuged at 1500 rpm for 15 min and the plasma and buffy coat were removed. The red cells were isolated and washed twice with 0.9% NaCl, and hemolysed with 1.5 volumes of ice-cold water. The ghost and intact cells were removed by centrifugation at 20 000 rpm for 30 min at 4°C. The pH of hemolysate was adjusted to 8.7 with solid Tris-HCl. The hemolysate was applied to the prepared Sepharose 4B-L-tyrosine-sulfanylamide affinity column equilibrated with 25 mM Tris-HCl/0.1 M Na_2SO_4 (pH 8.7). The affinity gel was washed with 25 mM Tris-HCl/22 mM Na_2SO_4 (pH 8.7). The human carbonic anhydrase (HCA-I and HCA-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 [33].

Total protein determination

The absorbance at 280 nm was used to monitor the protein in the column effluents. Quantitative protein determination was achieved by absorbance measurements at 595 nm according to Bradford [34], with bovine serum albumin standard.

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was performed in order to verify the purified enzyme. It was carried out in 12% and 3% acrylamide concentrations for the running and the stacking gel, respectively, containing 0.1% SDS according to Laemmli [35]. Sample (20 µg) was applied to the electrophoresis medium. Gel was stained for 1.5 h in 0.1% Coommassie Brillant Blue R-250 in 50% methanol and 10% acetic acid, then destained with several changes of the same solvent without the dye. The electrophoretic pattern was photographed (Figure 2).

CA Enzyme Assay

The CA enzyme activity was assayed by following the hydration of CO_2 according to the method described by Wilbur and Anderson [36]. CO_2 hydratase activity of enzyme was determined at room temperature in a veronal buffer (pH 8.6) with phenol-red as indicator and satured carbon dioxide solution as substrate in a final volume of 4.2 ml. The time (in seconds) taken for the solution to change from red to yellow was measured. The enzyme unit (EU) was calculated using the equation (t_0-t_c / t_c) where t_0 and t_c are the times for pH change of the non enzymatic and enzymatic reactions, respectively.

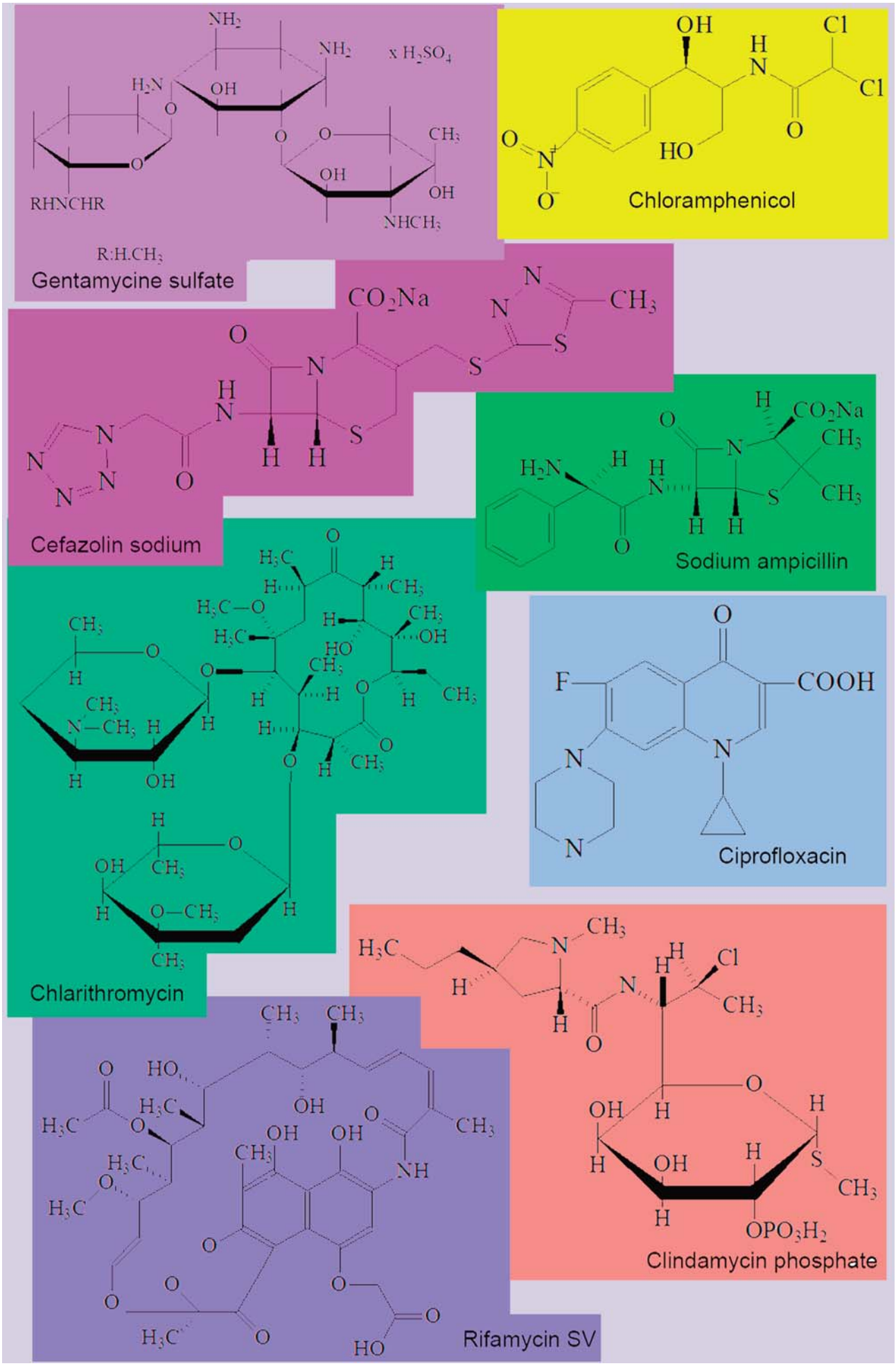

Figure 1. The structure of medical drugs.

***In vitro* studies**

For the inhibition Chlarithromycin, chloramphenicol, rifamycin SV, clindamycin phosphate, ciprofloxacin, gentamycin sulfate, sodium ampicillin and cefazolin sodium, at different concentrations were added to inhibit enzyme activity. Reaction mixture (4.0 ml) contained 2 ml phenol red, 1.5 ml CO_2 solution, 0.3 ml buffer solution, 0.1 ml inhibitor at various concentrations and 0.1 ml enzyme solution. Sensitivity of CA to antibiotics was examined by measuring CA activity in the presence of increasing concentrations of these medical drugs. CA activities with the related antibiotics were assayed by following the hydration of CO_2. Percent activity graphs were drawn from the results to find IC_{50} values at five different antibiotic concentrations, which show about 50% inhibition effect. In order to determine IC_{50} value, regression analysis graphs were drawn by using percent inhibition values by an Excell program on a computer. The inhibition concentrations causing up to 50% inhibition were determined from the graphs. CA activity without pesticides and inhibitors was accepted as 100% activity. Each inhibition effects were repeated at least three times. The structures of medical drugs used are shown in Figure 1.

***In vivo* studies**

Nine mice (*Mus musculus* diolecticus, white type) (25 ± 6 g) were selected for intramuscular administration of each drug. Mice used for *in vivo* studies have been kept under special conditions (in a windowless room, 22°C, with light on for 12 h) for 1 month. A group of three mice were included in the study for a control group, which is not subject to any drug administration. Drug dosage for mice was calculated from suggested dose for humans in mg/kg. Ciprofloxacin (3.2 mg/kg), chlarithromycin (7.14 mg/kg), rifamycin SV (3.56 mg/kg), gentamycin sulfate (3.2 mg/kg), clindamycin phosphate (2.143 mg/kg), chloramphenicol (12.5 mg/kg), sodium ampicillin (17.12 mg/kg) and cefazolin sodium (106.25 mg/kg) were injected intramuscularly into each mouse. For each drug mice were sacrificed by using cervical dislocation method at 2, 4 and 6 h time points of the drug administration. Blood samples were taken out from each mouse at these points after injection. Blood was collected into the test tubes containing EDTA and plasma was separately by centrifugation. Hemolysate was prepared immediately and stored at -80°C during the preparation of analysis.

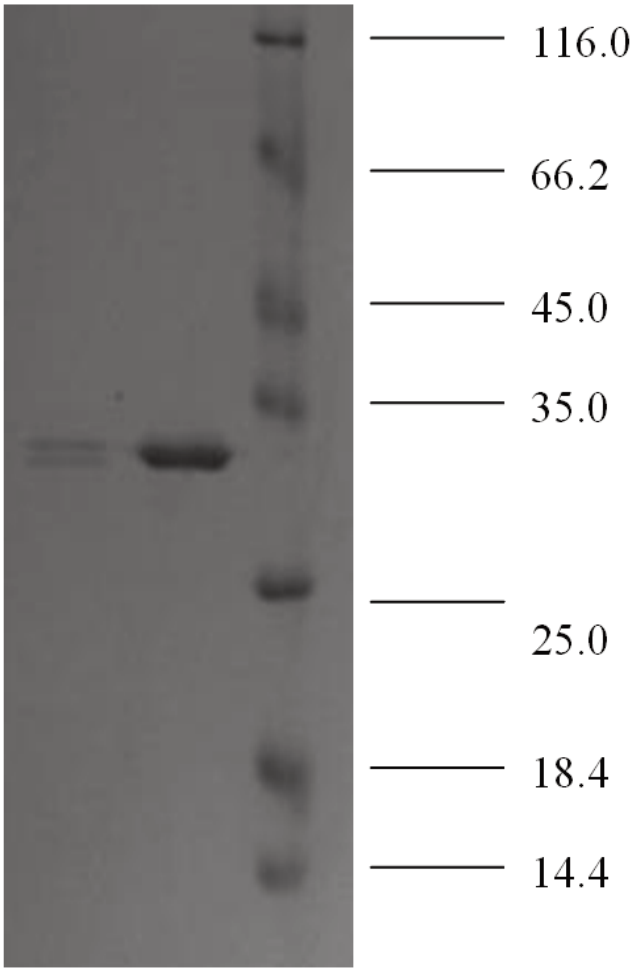

Figure 2. SDS-PAGE of human carbonic anhydrase I and II. The pooled fractions from affinity chromatography (sepharose-4B, L-tyrosine, sulfanilamide) was analyzed by SDS-PAGE (12% and 3%) and revealed by Coomassie Blue staining. Experimental conditions were described in the method. Lane 3 contained 3 µg of various molecular-mass standards: β-galactosidase, (116.0), bovine serum albumin (66.2), ovalbumin (45.0), lactate dehydrogenase, (35.0), endonuclease (25.0), β-lactoglobulin (18.4), lysozyme (19.5). Thirty microgram of purified human carbonic anhydrase I (lane 2) and I (lane 1) migrated with a mobility corresponding to an apparent M_r 33.0 kDa.

Statistical analysis

Statistical analysis was performed using SPSS program. Analysis of variance, Tukey HSD was used when more than two groups were compared. Data are presented as mean ±S.D. The values $P < 0.05$ were considered significant.

RESULTS

In vitro studies

Human erythrocytes CA I and II were purified by using the affinity gel with the elution buffers of 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. Purification of the isoenzymes was confirmed with SDS-polyacrylamide gel electrophoresis (Figure 2). Chlarithromycin, chloramphenicol, rifamycin SV, clindamycin phosphate, ciprofloxacin, gentamycin sulfate and sodium ampicillin and cefazolin sodium were chosen for the investigation of inhibition or activation effects. To show inhibition effects, activity % values of HCA-I and HCA-II for five different concentrations of each medical drug were determined. Carbonic anhydrase activity without a medical drug was accepted as 100% activity. For the drugs exhibiting an inhibition effect, the inhibitor concentrations causing up to 50% inhibition (IC_{50}) were determined from the regression analysis graphs. IC_{50} values obtained for HCA-I and HCA-II are shown in Table 1. From *in vitro* studies, HCA-I and HCA-II were inhibited by all medical drugs gradually. Sodium ampicillin was most effective inhibitors for HCA-I and HCA-II. The obtained IC_{50} values of sodium ampicillin were 0,00163 mM on HCA-I and 0,00114 mM on HCA-II. Cefazolin sodium had minimum effect on HCA-I and HCA-II (0.00358 mM and 0.00308 mM, respectively) when the compare to other antibiotics. Gentamycin sulfate had inhibition effect on each enzyme. As seen in Table 1, IC_{50} values of gentamycin sulfate was found to be 0.00235 mM and 0.00253 mM.

Table 1. Inhibition effects of drugs on carbonic anhydrase.

	IC_{50} (mM)	
Drug	HCA-I	HCA-II
Ciprofloxacin	0.00292	0.00212
Chlarithromycin	0.00352	0.00253
Rifamycin SV	0.00252	0.00102
Gentamycin sulfate	0.00235	0.00253
Clindamycin Phosphate	0.00341	0.00189
Chloramphenicol	0.00472	0.00208
Sodium ampicillin	0.00163	0.00114
Cefazolin sodium	0.00358	0.00308

In vivo studies

For *in vivo* studies, mouse erythrocytes CA was determined at three time points, namely 2, 4 and 6 h after injection. The results of *in vivo* effects of each antibiotic are presented in Table 2. The mean ±S.D. values of carbonic anhydrase activities in the test group and control group were compared in Figure 3. Our data showed that, there is no correlation between the *in vivo* and *in vitro* effects of each antibiotic, except sodium ampicillin and cefazolin sodium. Ciprofloxacin (3.2 mg/kg), chlarithromycin (7.14 mg/kg), rifamycin SV (3.56 mg/kg), gentamycin sulfate (3.2 mg/kg) and clindamycin phosphate (2.143 mg/kg) exhibited statistically significant activation effect for the mouse erythrocytes CA activity ($p>0.05$). However, CA activity after sodium ampicillin and cefazolin sodium administration showed statistically significant decrease at 4 h of drug application *in vivo* (p: 0.014 and 0.016, respectively). For ciprofloxacin, chlarithromycin and rifamycin SV, the activity of the control, which did not contain any drug, was determined as 13.46±2.90 EU. Then the drug injection was performed on the control groups intraperitoneally. The activities of the groups after the drug injection were measured at 2, 4 and 6 h, and corresponding activities were found to be 8.25±1.35a, 51.45±4.14b and 8.25±1.34a for ciprofloxacin; 24.61±7.00a, 46.90±2.74b and 22.24±3.74a for chlarithromycin; 14.08±1.54a,

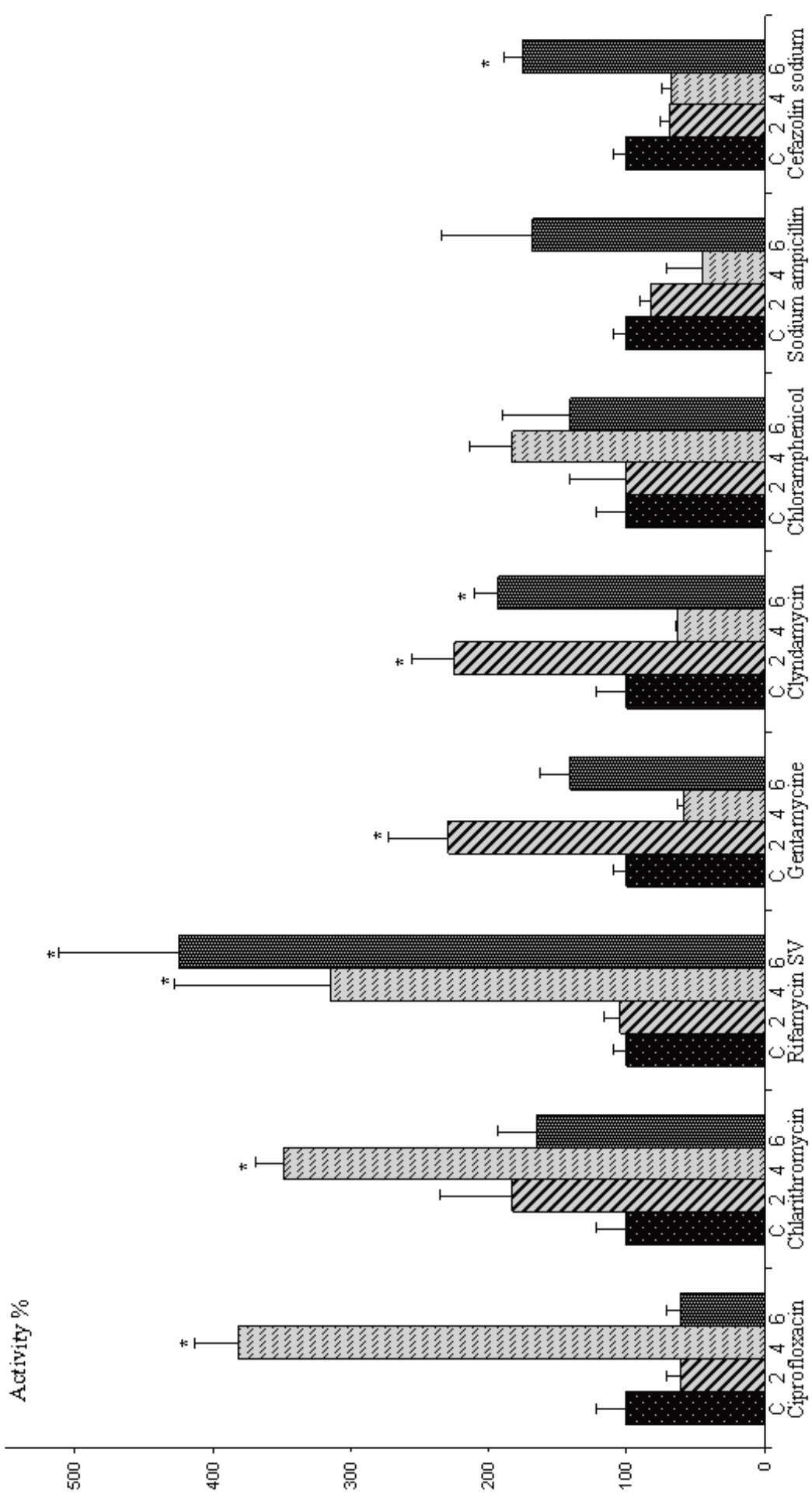

Figure 3. In vivo effects of chlarithromycin, chloramphenicol, rifamycin SV, clindamycin phosphate, ciprofloxacin, gentamycin sulfate, sodium ampicillin and cefazolin sodium, on mouse erythrocyte carbonic anhydrase activity. Values are mean ± S.D. for n = 3 independent experiments per group of CA activity measured after the drug administration. Analysis of variance, TUKEY, was used when more than two groups were compared. The values $P < 0.05$ were considered significant. (C, control group; 2, 4, 6 h, CA activity % after the drug administration; * , the statistically significant data [4 h (P, 0.000) for ciprofloxacin, 4 h (P, 0.000) for chlarithromycin, 4 h (P, 0.000) and 6 h (P, 0.049) for rifamycine SV, 2 h (P, 0.01) for gentamycine sulfate, 2 h (P, 0.000) and 6 h (P, 0.003) for clindamycin phosphate, 6h (P, 0.000) for cefazolin sodium, sodium ampicillin 4 h (p:0.015).

Table 2. The in vivo effects of chlarithromycin, chloramphenicol, rifamycin SV, clindamycin phosphate, ciprofloxacin, gentamycin sulfate and sodium ampicillin and cefazolin sodium on erythrocytes carbonic anhydrase activity. Data represent the mean ± S.D. n = 3 denotes the number of mice used in each experiment. (Tukey HSD: P<0.05)

Drugs (mg/ml)	Time (h)	Number (n)	CA Activity (mean±SD)	P Value
Ciprofloxacin	Control	3	13.4 ± 2.9	-
	2	3	8.2 ± 1.3	0.163
	4	3	51.4 ± 4.1	P<0.05
	6	3	8.2 ±1.3	0.163
Chlarithromycin	Control	3	13.4 ± 2.9	-
	2	3	24.6 ± 7.0	0.060
	4	3	46.9 ± 2.7	P<0.05
	6	3	22.2 ± 3.7	0.150
Rifamycin SV	Control	3	13.4 ± 2.9	-
	2	3	14.0 ± 1.5	1.000
	4	3	42.4 ± 15.1	P<0.05
	6	3	57.1 ± 11.6	P<0.05
Gentamycin sulfate	Control	3	8.2 ± 0.8	-
	2	3	19.0 ± 3.6	P<0.05
	4	3	4.9 ± 0.3	0.256
	6	3	11.7 ± 1.6	0.241
Clindamycin phosphate	Control	3	13.4 ± 2.9	-
	2	3	30.2 ± 4.1	P<0.05
	4	3	8.5 ± 0.1	0.216
	6	3	25.9 ± 2.3	P<0.05
Chloramphenicol	Control	3	13.4 ± 2.9	-
	2	3	13.5 ± 5.4	1.00
	4	3	24.6 ± 4.0	0.94
	6	3	18.9 ± 6.6	0.559
Sodium ampicillin	Control	3	8.2 ± 0.8	-
	2	3	6.8 ± 0.7	0.924
	4	3	3.7 ± 2.1	P<0.05
	6	3	13.9 ± 5.3	0.167
Cefazolin sodium	Control	3	8.2 ± 0.8	-
	2	3	5.7 ± 0.5	0.020
	4	3	5.6 ± 0.5	P<0.05
	6	3	14.4 ± 1.1	P<0.05

42.47±15.12b and 57.11±11.68bc for rifamycin SV, respectively (Table 2). Our data showed that ciprofloxacin, chlarithromycin and rifamycin SV increased the activity of CA in *in vivo* studies.

For gentamycin sulfate and clindamycin phosphate, it was observed that the activity of the control, which did not contain any drug, was determined as 8.29±0.8 EU and 13.46±2.90 EU, respectively. The activities of the groups after the drug injection were measured at 2, 4 and 6 h, and corresponding activities were found to be 19.01±3.61c, 4.91±0.34a and 11.75±1.69b for gentamycin sulfate and 30.27±4.16b, 8.55±0.15a and 25.92±2.31b for clindamycin phosphate, respectively. The highest activation values were found for 2 h after injection of these antibiotics and 6 h after injection of clindamycin phosphate.

DISCUSSION

Many compounds alter the activity of an enzyme by combining with it in a way that influences the binding of substrate and/or its turnover number. Substances that reduce an enzyme activity in this way are known as inhibitors. Many inhibitors are substances that structurally resemble their enzyme's substrate but either do not react, or react very slowly, compared with the substrate. Such inhibitors are commonly used to probe the chemical and conformational nature of a substrate-binding site as part of an effort to elucidate the enzyme's catalytic mechanism [37]. Many chemicals at relatively low dosages affect the metabolism of biota by altering normal enzyme activity, particularly inhibition of a specific enzyme [38]. The effects can be dramatic and systemic [39]. Indeed, CA isozymes are important enzymes in metabolism because they regulate pH in most tissues. CA inhibitors vary according to their affinity of binding to a particular CA isozyme, potency (IC_{50}) for inhibiting that isozyme, and physicochemical properties, which can influence their tissue distribution and scope of activity [20].

It has been reported that the most common inhibitors for CA are several medical drugs [20,21]. In addition, some sulfonamide derivatives, antiepileptic drugs and melatonin have been used by investigations for inhibition of CA activity *in vivo* and *in vitro* [20,40,41]. However, there was not much information available on inhibition of human CA enzymes by antibiotics. In the present study, the investigation of effects of certain medical drugs on human erythrocytes carbonic anhydrase I and II isozymes was proposed.

Antibiotics are an important group of pharmaceuticals in today's medicine. In this study, macrolid, amphenicol, aminoglicoside, penicillin, and beta-lactam derivatives antibiotics were chosen. The chosen of different groups of antibacterial antibiotics; chlarithromycin, chloramphenicol, rifamycin SV, clindamycin phosphate, ciprofloxacin, gentamycin sulfate and sodium ampicillin and cefazolin sodium were chosen for the investigation of inhibition or activation effects. IC_{50} values obtained for in vitro studies of HCA-I and HCA-II are shown in Table 1.

The antibiotics employed in the present study, which are commonly used in therapy, showed inhibitory effects on the activity of human erythrocytes carbonic anhydrase isoenzymes I and II to various degrees. Sodium ampicillin was most effective inhibitor for HCA-I and HCA-II. The obtained IC_{50} values of sodium ampicillin were 0.00163 mM on HCA-I and 0.00114 mM on HCA-II. Beydemir et al. [20] also reported that HCA-I and HCA-II were inhibited by sodium ampicillin. Cefazolin sodium had minimum effect on HCA-I and HCA-II (0.00358 mM and 0.00308 mM, respectively) when the compare to other antibiotics. Gentamycin sulfate had inhibition effect on each enzyme. IC_{50} Values of gentamycin sulfate was found to be 0.00235 mM and 0.00253 mM. However, it is reported that, HCA-I and HCA-II were inhibited at gentamycin sulfate concentration up to 2 mM, and activated at up to 4 mM [21].

In order to determine the inhibition statue of these drugs on a living system, the effects of same antibiotics on CA enzyme activity of mouse erythrocytes was investigated *in vivo*. For *in vivo* studies, mouse erythrocytes CA was determined at three time points, namely 2, 4 and 6 h after injection. The results of *in vivo* effects of each antibiotic are presented in Table 2. Our data showed that, there is no correlation between the *in vivo* and *in vitro* effects of each antibiotic, except penicillin and beta-lactam derivatives sodium ampicillin and cefazolin sodium, respectively. Indeed, other class of antibiotics, ciprofloxacin, chlarithromycin, rifamycin SV,

gentamycin sulfate and clindamycin phosphate were exhibit statistically significant activation effect for the mouse erythrocytes CA activity ($p>0.05$). However, CA activity after sodium ampicillin and cefazolin sodium administration showed statistically significant decrease at 4 h of drug application *in vivo* (p: 0.014 and 0.016, respectively). In addition, ciprofloxacin, chlarithromycin and rifamycin SV increased the activity of CA in *in vivo* studies.

For gentamycin sulfate, aminoglicoside derivative antibiotics, it was observed that while the activity of the mCA was increased at 2 h, the activity decrease 4 h, after drug injection. However, the highest activation values were found for 2 h after injection of these antibiotics and 6 h after injection of clindamycin phosphate. Our results were supported by a study which is showed that rat erythrocytes CA activity inhibited by gentamycin sulfate after 1 h and 3 h drug administration [21].

Chloramphenicol had inhibition effect on CA in *in vitro* studies, whereas it does not show any effect of the enzyme *in vivo.* Actually, mouse CA activity was activated by chloramphenicol 4 h after drug administration, but it is not statistically significant. On the contrary, one of the *in vivo* results supported *in vitro* data. Penicillin and beta-lactam derivative antibiotics name of sodium ampicillin and cefazolin sodium decreased the activity of mCA after 4 h drug injection. This inhibition was ststistically significant ($p<0.05$).

It is generally recognized that CA controls the bulk of carbon dioxide exchange between 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 in a great number of secretory activities including fluid movements [42]. Since CA is a very important enzyme for the body, the inhibition effects of these medical drugs should be considered for not only the erythrocyte HCA-I and HCA-II isozymes but also all CA isozymes. For example, in two recent studies, liver total CA (CAI, CAII, CAIII, CAIV) activity was diminished in streptozotocin-induced diabetic rats [43,44]. These studies also demonstrated gluconeogenesis and ureagenesis with an increase in isozyme CA-V activity in hepatocytes [43,45]. They have shown pH disequilibrium in the rat liver that could be explained by the changes in CA activity. In addition, many of the side-effects observed are probably due to inhibition of CA isozymes. For example, the respiratory acidosis is probably responsible for some of the side reactions observed during acetazolamide therapy such as fatigue, headache, taste sensations and for the distress in the chronic lunger [46]. For each drugs, suggestion dosage in medical treatment is nearly suitable for carbonic anhydrase enzyme activity, except sodium ampicillin and cefazolin sodium from *in vivo* studies.

According to our results (Figure 3), the use of ciprofloxacin, chlarithromycin, rifamycin SV, gentamycin sulfate and clindamycin phosphate at the suggested concentration increased the activities of CA enzyme. However, the use of sodium ampicillin and cefazolin sodium at the suggested concentration inhibited the activities of CA enzyme. Therefore, the use of sodium ampicillin and cefazolin sodium cannot be recommended for this enzyme.

Differential sensitivity of organism CAs might be depending on a number of factors. It is possible that differences in inhibition and activation, rooted in the differences in binding affinity of the antibiotics or metabolyzing product to the enzyme. Consequently, carbonic anhydrase enzyme activity was inhibited by the use of each drug for *in vitro* studies, because of the pure enzyme is more sensitive for the outside effects. However, these antibiotics had not inhibition effects on mouse erythrocytes carbonic anhydrase

except sodium ampicillin and cefazolin sodium. On the contrary, these antibiotics had activation effect on mouse carbonic anhydrase activity from *in vivo* studies. Therefore, ciprofloxacin, chlarithromycin, rifamycin SV gentamycin sulfate and clindamycin phosphate can be recommended for the use of medical treatment, for this enzyme.

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