

ORIGINAL ARTICLE

Antipsychotic agents screened as human carbonic anhydrase I and II inhibitors

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Abstract

The antipsychotic drugs currently used to treat schizophrenia can be divided into two distinct classes, *typical* and *atypical* antipsychotics. Many drug molecules are enzyme inhibitors that bind reversibly or irreversibly to their target through intermolecular interactions. That's why enzyme inhibition studies are an important issue for drug design and biochemical applications. In this study, *in vitro* inhibition effect of some antipsychotic drugs on the purified carbonic anhydrase (CA) I and II isoenzymes were investigated by using CO₂ as a substrate. CA I and II were purified from human erythrocytes by a simple one step procedure using Sepharose 4B-L-tyrosine-sulfonamide affinity column. The results showed that all the drugs inhibited the cytosolic carbonic anhydrases enzyme activity in a concentration-dependent fashion. Among the studied drugs, aripiprazole and pramipexole were found to be the most active one for hCA I (IC₅₀: 3.64 and 5.37 µM) and hCA II (IC₅₀: 4.16 and 4.81 µM) activity, respectively.

Keywords

Antipsychotic drugs, carbonic anhydrase, inhibition, purification

History

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Introduction

Carbonic anhydrase (EC: 4.2.1.1; CA) is a family of metalloenzymes that catalyse the conversion of CO₂ to HCO₃⁻ and H⁺, being involved in many physiologic processes (Supuran, 2008). CA isoforms are found in a variety of tissues where they participate in several important biological processes such as acid-base balance homeostasis, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis, electrolyte secretion, and tumorigenesis among others (Beydemir & Gulcin, 2004; Bottcher *et al.*, 1994; Casey, 2006; Hilvo *et al.*, 2008; Sly & Hu, 1995; Supuran & Scozzafava, 2002, 2007; Supuran, 2010; Zhenyan *et al.*, 2006). Many CA isozymes are well known as therapeutic targets with the potential inhibition or activation effects for the treatment of disorders such as oedema, glaucoma, obesity, cancer, epilepsy and osteoporosis (Casey, 2006; Sly & Hu, 1995; Supuran & Scozzafava, 2002; Supuran, 2010). CA which is a widespread metallo-enzyme has previously been purified and characterized from many living organisms including animals (Beydemir & Gulcin, 2004; Bottcher *et al.*, 1994; Zhenyan *et al.*, 2006). The isozymes of CA play important roles in different tissues (Bulbul *et al.*, 2003; Supuran *et al.*, 2001). The similarities of CAs from various sources have been determined from their

crystal structures (Huang *et al.*, 1998). It is known that carbonic anhydrase has been purified many times from different organisms and the affects of various chemicals, pesticides and drugs on its activity have been investigated (Arslan *et al.*, 2011; Celik *et al.*, 1996; Coban *et al.*, 2009; Ekinci *et al.*, 2007; Gervais & Tufts, 1999; Senturk *et al.*, 2011, 2012; Vitale *et al.*, 1996).

The antipsychotic drugs currently used to treat schizophrenia can be divided into two distinct classes, *typical* or first generation antipsychotics (FGAs) and *atypical* or second generation antipsychotics (SGAs) (Carpenter *et al.*, 1998; Miyamoto *et al.*, 2005; Sawa & Snyder, 2002). The distinction between these two drug classes is based on the time of introduction to the market, FGAs preceding SGAs, and their receptor binding profiles. FGAs block DA D2 receptors, while SGAs have antagonist activity at both D2 and 5HT₂ receptors (Creese *et al.*, 1976; Richtand *et al.*, 2007; Roth *et al.*, 2004; Seeman *et al.*, 1976). Of greatest importance, however, is the ability, albeit limited, of SGAs to treat the negative symptoms of schizophrenia that is coupled with a lower risk of developing the tardive dyskinesias associated with FGA use (Carpenter *et al.*, 1998; Miyamoto *et al.*, 2005; Sawa & Snyder, 2002).

Our groups recently investigated the interaction of two mammalian CA isozymes with several compounds, such as analgesic drugs and a series of anabolic compounds, pesticides, macro cyclic thiocrown ethers, cattle drugs and coumarin derivatives (Arslan *et al.*, 2012; Cicek *et al.*, 2012; Gencer *et al.*, 2012a, b; Gokce *et al.*, 2012; Karatas *et al.*, 2013).

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In literature, there are so many inhibition studies of CA isozymes with sulphonamides compounds, but there are not enough studies related to the inhibition of CA isozymes, especially by antipsychotic drugs. In this present study, we tried to examine the *in vitro* inhibitory effects of 25 antipsychotic drugs on the purified cytosolic CA I and II isoenzymes from human erythrocytes.

Materials and methods

Materials

Sepharose 4B, L-tyrosine, sulphonamide, protein assay reagents and chemicals for electrophoresis were obtained from Sigma Chemical Co. All other chemicals used were of analytical grade and obtained from either Sigma or Merck. All drugs were provided by the local pharmacy.

CA enzyme assay

Cytosolic CA I and II isozymes were purified from human erythrocytes by a simple one step procedure using Sepharose 4B-L-tyrosine-sulphonamide affinity column (Arslan *et al.*, 1996). CA activity was measured based on the determination

of the time required for the pH to decrease from 10.0 to 7.4 due to CO₂ hydration as described by Maren (1960). The assay solution was 0.5 M Na₂CO₃/0.1 M NaHCO₃ (pH 10.0) and Phenol Red was added as the pH indicator. CO₂-hydratase activity (enzyme units (EU)) was calculated by using the equation $t_0 - tc/tc$ where t_0 and tc are the times for pH change of the non-enzymatic and the enzymatic reactions, respectively.

In vitro inhibition studies

For the inhibition studies of antipsychotic drugs, different concentrations of these compounds were added to the enzyme. Activity percentage values of CA for different concentrations of each drug were determined by regression analysis using Microsoft Office 2000 Excel. CA enzyme activity without a drug solution was accepted to be 100%.

Preparation of hemolysate and purification of CA isozymes from human red blood cells

Blood samples (25 mL) were taken from healthy human volunteers. The blood samples were anti-coagulated with acid-citrate-dextrose, centrifuged at 5000 rpm for 10 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then hemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 15 000 rpm for 30 min at 4 °C

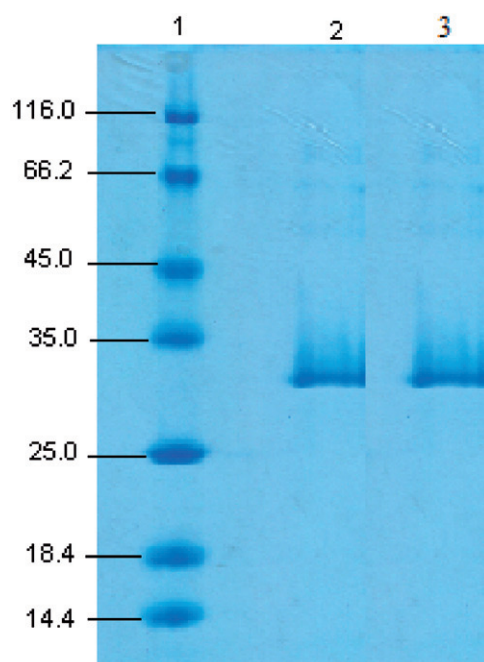


Figure 1. SDS-PAGE of human carbonic anhydrase isozymes. The pooled fractions from affinity chromatography (Sepharose 4-B, L-tyrosine, sulphonilamide) was analysed by SDS-PAGE (%12 and %3) and revealed by Coomassie Blue staining. Experimental conditions were as described in the method. Lane 1 contained 5 µg of various molecular mass standards: β-galactosidase (116.0), bovine serum albumin (66.2), ovalbumin (45.0), lactate dehydrogenase (35.0), Restriction endonuclease (25.0), β-lactoglobulin (18.4), lysozyme (14.4). 100 microgram of purified human carbonic anhydrase I and II (lane 2 and lane 3) migrated with a mobility corresponding to an apparent Mr 33.0 kDa.

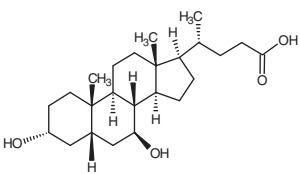
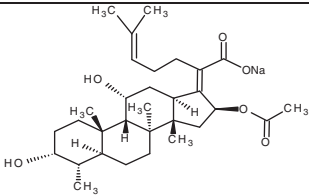
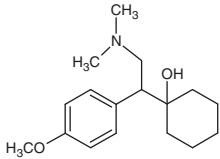
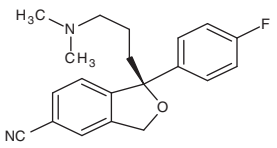
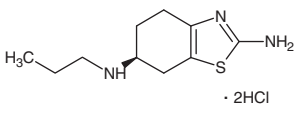
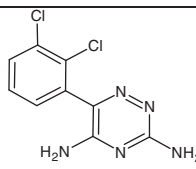
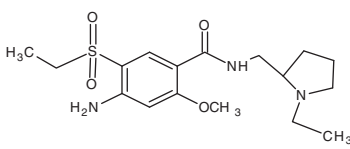
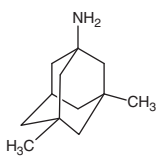
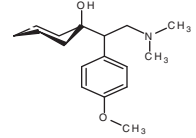
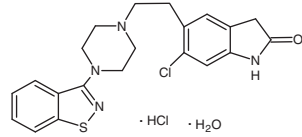
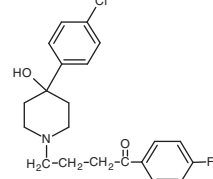
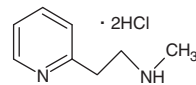
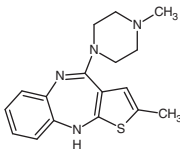
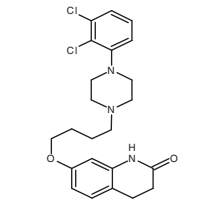
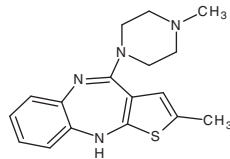
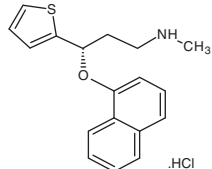
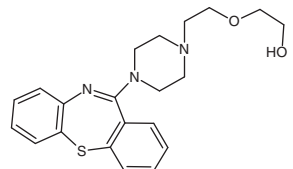
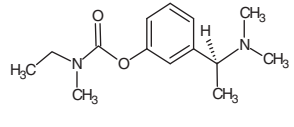
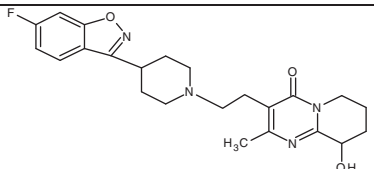
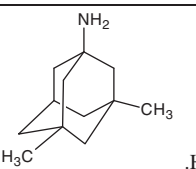
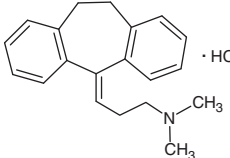
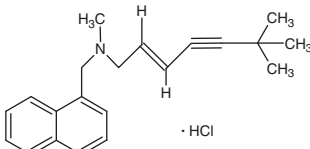
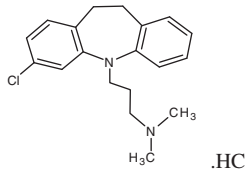
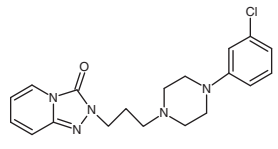
Table 2. The IC₅₀ values of antipsychotic drugs.

No.	Drug substance	hCA I IC ₅₀ (µM)	hCA II IC ₅₀ (µM)
1	Memantine hydrochloride	132.92	142.93
2	Clomipramine hydrochloride	247.94	278.36
3	Amitriptyline hydrochloride	205.75	260.34
4	Lamotrigine	811.20	977.30
5	Venlafaxine	596.71	742.53
6	Sodium fusidate	3868.00	3390.00
7	Haloperidole	95.69	139.58
8	Duloxetine hydrochloride	536.00	278.35
9	Ursodeoxycholic acid	2067.00	2329.00
10	Rivastigmine	28.25	27.71
11	Pramipexole	5.37	4.81
12	Olanzapine	131.59	119.61
13	Paliperidone	61.10	94.51
14	Escitalopram	420.10	637.25
15	Bethahistine dihydrochloride	376.13	327.81
16	Escitalopram	240.66	290.92
17	Aripiprazole	3.64	4.16
18	Trazodone hydrochloride	308.63	393.58
19	Terbinafine hydrochloride	2867.00	3288.00
20	Ziprasidone	315.64	385.41
21	Amisulpride	503.78	1536.00
22	Olanzapine	224.88	301.13
23	Memantine	158.49	197.75
24	Quetiapine	1472.00	1778.00
25	Venlafaxine	476.63	528.54

Table 1. Summary of the purification of human carbonic anhydrase I and II.

Step	Volume (ml)	Activity (U/ml)	Total activity (U)	Protein amount (mg/ml)	Total protein (mg)	Specific activity (U/mg)	Overall yield %	Overall purification (fold)
Hemolysate	25	41.33	1033.25	1.9980	49.9500	20.68	100.00	–
Affinity chromatography	2	83.08	166.16	0.0153	0.0306	5430.06	16.08	262.57

Table 3. Chemical structures of antipsychotic drugs.

		
Ursodeoxycholic acid	Sodium fusidate	Venlafaxine
		
Escitalopram	Pramipexole · 2HCl	Lamotrigine
		
Amisulpride	Memantine	Venlafaxine
		
Ziprasidone	Haloperidol	Betahistine dihydrochloride · 2HCl
		
Olanzapine	Aripiprazole	Olanzapine
		
Duloksetin hydrochloride .HCl	Quetiapine	Rivastigmine
		
Paliperidone	Memantine hydrochloride .HCl	Amitriptyline HCl
		
Terbinafine hydrochloride · HCl	Clomipramine HCl .HCl	Trazodone hydrochloride · HCl

and the pH of the hemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 mL hemolysate was applied to the affinity column comprising Sepharose-4B-L-tyrosine-sulfonamide gel equilibrated with 25 mM Tris-HCl/0.1 M Na₂SO₄ (pH 8.5) (Arslan *et al.*, 1996). The affinity gel was washed with 50 mL of 25 mM Tris-HCl/22 mM Na₂SO₄ (pH 8.5). The hCA isozymes were then eluted with 0.1 M NaCl/25 mM Na₂HPO₄ (pH 6.3) and 0.1 M CH₃COONa/0.5 M NaClO₄ (pH 5.6), which recovered hCA-I and hCA-II respectively. Fractions of 3 mL were collected and their absorbance measured at 280 nm.

Results and discussion

In this study, CA I and II isozymes from human erythrocytes were purified by a simple one step procedure using Sepharose 4B-L-tirozin-sulfonamide affinity column and purity of the enzyme was confirmed by SDS-PAGE (Figure 1). The overall purification gave CA in a yield of 16.08% with a specific activity of 5430.06 EU/mg proteins and the overall purification was 262.57-fold (Table 1). Results are listed in Table 2, in terms of molarity of the tested drugs causing a 50% reduction of the enzymatic activities. The results showed that all the drugs inhibited the enzyme activity in a concentration-dependent fashion. The inhibition values against CAs were given in the Table 2. It is determined that the inhibition values are in between 5.37–3868.00 µM for hCA I and 4.81–3390.00 µM for hCA II. As shown in Table 2, aripiprazole and pramipexole were found to be the most active one for hCA I (IC₅₀: 3.64 and 5.37 µM) and hCA II (IC₅₀: 4.16 and 4.81 µM) activity, respectively.

In literature, it has been reported that IC₅₀ values of sodium ampicillin were 0.385 mM on hCA-I and 0.774 mM on hCA-II (Beydemir *et al.*, 2000). Ozensoy *et al.* (2008) reported that cefuroxime axetil for hCA I and hCA II isozymes as IC₅₀ value of 2.92×10^{-5} and 2.12×10^{-5} mM, respectively. Recently, our group determined that the IC₅₀ concentrations of dexketoprofen trometamol and dexamethasone sodium phosphate on hCA I were 683 and 4250 µM and for hCA II 950 and 6200 µM respectively (Gokce *et al.*, 2012).

Puscas *et al.* (2001) reported that indomethacin, *in vitro* and *in vivo*, induces an increase in erythrocyte CA I and CA II activity. In humans, an increase or decrease in erythrocyte CA II activity is correlated with an increase or decrease in gastric acid secretion. Indomethacin is not only an activator of CA but also antagonizes the affect of acetazolamide, a specific inhibitor of this enzyme. Many drug side effects may be considered to result from CA isozyme inhibition. For example, respiratory acidosis is probably the cause of some side effects observed during acetazolamide therapy, such as fatigue, headache, altered taste sensations and distress (Thomsen *et al.*, 2000). Measurement of the CO₂ hydratase activity of CA-I and CA-II requires specific inhibitors or separation of the isozymes. It is difficult to study the factors and conditions that affect CA activity because standard CA activity assays have serious limitations. Therefore, estimate of the CA-I and CA-II level in erythrocytes are complicated by the pronounced differences in enzymatic activity of CA-I and CA-II (Nishita *et al.*, 2005).

Many drug molecules are enzyme inhibitors that bind reversibly or irreversibly to their target through intermolecular interactions. That is why enzyme inhibition studies are an important issue for drug design and biochemical applications (Alim & Beydemir, 2012; Demir *et al.*, 2012a, b; Sayin *et al.*, 2012; Sonmez *et al.*, 2011). This study is the first report on the inhibition of cytosolic carbonic anhydrases by various antipsychotic drugs. Among the studied drugs, at low concentrations, aripiprazole and pramipexole had the strongest *in vitro* inhibitory effects on hCA I (IC₅₀: 3.64 and 5.37 µM) and hCA II (IC₅₀: 4.16 and 4.81 µM) activity. As stated earlier, many drug side effects may be considered to result from CA isozyme inhibition. Uncontrolled usage of these mentioned drugs could cause serious adverse effects and could be deleterious to health. For this reason, these drugs must be used carefully and the dosage should be closely monitored to decrease their side effects. Depending on our data, it is understood that usage of these drugs must be well-determined as they might have serious side effects on CA enzymes which may result in the disruption of acid-base balance and salt transport. Consequently, including the outcome of this present study results, further *in vivo* studies could help to reveal the inhibition mode of these drugs on cytosolic carbonic anhydrases. Detailed information regarding the structure of all drugs presented in this letter can be found in the supplementary data provided (as shown in Table 3).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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