THE INVESTIGATION OF THE INTERACTION OF SEVERAL ANTIPSYCHOTIC DRUGS WITH HUMAN CHOLINESTERASE ENZYMES

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ABSTRACT

Phenothiazines and butyrophenones are one of the important antipsychotic group of drugs. They have been utilized for many years for treatment of schizophrenia and other psychotic disorders. They are the main constituents of the first generation, also referred as typical, antipsychotic drugs. They are known for their potential to antagonize dopamine D_2 receptors. With respect to their structures, they are able to cross the blood brain barrier and interact with various types of receptors. In order to investigate their potential to interact with cholinesterase enzymes, within this research, we have designed a series of experiments. Although, earlier studies have shown that some of those compounds have the potential to inhibit cholinesterase enzyme, for the first time, we have evaluated their potential to inhibit human isoforms of acetylcholinesterase and butyrylcholinesterase. The results strongly pointed out that phenothiazines are selective and potent inhibitors of the human butyrylcholinesterase enzyme.

Key words: Phenothiazines, Butyrophenones, Human cholinesterase inhibitory potential

INTRODUCTION

Moreover, they have ability to act like quinidine and show effect on cardiac system (Dougherty and Marraffa 2014; Jaszczyszyn et al. 2012). It was shown that these compounds are not specific on dopaminergic system, since they can also interact with some other receptors within the central nervous system (Darvesh et al., 2010). In particular, some of them are known to inhibit cholinesterase enzymes that have been investigated on the cholinesterase enzymes obtained from various species but human (Darvesh et al., 2010). For instance some phenothiazine derivatives such as chlorpromazine and ethopropazine were reported to be BuChE inhibitor molecules, although there are used to treat schizophrenia (Darvesh et al., 2005). This selectivity was attributed both to the structural organization of chlorpromazine and ethopropazine and derivatively smaller and different organization of the active side of AChE (Darvesh et al., 2013). However, the derivatives of these compounds synthesized were shown

to have different selectivity in a such a way that the N-10-amide derivatives of phenothiazines were found the inhibitors of BuChE, while there carbamate analogs were found to show selectivity towards AChE (Darvesh et al., 2010). They block dopaminergic receptors by interacting to D_1 and D_2 receptors (Jaszczyszyn et al., 2012; Mayoclinic, 2016; Sudeshna & Parimal, 2010).

Although, the cholinesterase inhibitors potential of some antipsychotic drugs are known , a detailed study has not been accomplish so far to show their interaction with human cholinesterase enzymes therefore, within this research, we have utilized chlorpromazine, trifluperazine, perphenazine, thioridazine and haloperidol antipsychotic agents and measured their IC_{50}s to inhibit human cholinesterase enzymes.

MATERIALS AND METHODS

Chlorpromazine, trifluperazine, perphenazine, thioridazine and haloperidol are obtained from Sigma Aldrich (CA, USA). Their purity was more than 99% as stated on their labels, therefore, no other purification was conducted on the compounds.

Determ*ination of AChE and BChE inhibitory activities*

The modified spectrophotometric method of Ellman (1961) was used to determine of AChE and BuChE inhibitory activities of 5 compounds (Chlorpromazine, Trifluperazine, Perphenazine, Thioridazine and Haloperidol) (Ellman, Courtney, Andres, & Featherstone, 1961). The enzymes used for cholinesterase activity studies were, human recombinant AChE (HuAChE) (Sigma) and human recombinant BuChE (Sigma).

Acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5, 5´-Dithio-bis (2-nitrobenzoic) acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. Other reagents and conditions, briefly, 50 mM Tris HCl buffer (pH 8.0), 6.8 mM DTNB, 2 µl of sample solutions and 10 µl of AChE/BChE solution were added in a 96-well microplate. The reaction was then initiated with the addition of 10 µl of acetylthiocholine iodide/butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate reader (Varioskan Flash, Thermo Scientific, USA) and incubated for 15 min at 27°C. The measurements and calculations

were evaluated by using SkanIt Software 2.4.5 RE for Varioskan Flash software. Percentage of inhibition of AChE and BuChE was determined by comparison of rates of reaction of samples relative to blank sample (methanol) using the formula (E-S)/E x 100, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Donepezil hydrochloride, rivastigmine (Sigma-Aldrich,USA), and Galantamine hydrobromide (from Lycoris sp. Sigma-Aldrich, St. Louis, MO, USA) used as reference compounds. The IC_{50} value obtained for each test compound with standard compounds (Ercetin, Senol, Erdogan Orhan, & Toker, 2012; Gulcan et al., 2014).

RESULT AND DISCUSSION

The potential of test compounds concomitant to the reference molecules to inhibit human cholinesterase enzymes were tested employing the Ellman's method as described in materials and method section. Accordingly, the results obtained are shown in Table 1. The activity of reference molecules was found parallel to the literature (Darvesh et al., 2013). Rivastigmine was found BuChE selective, while galantamine and donepezil showed selectivity towards AChE. Within the test conditions employed donepezil was found to be most potent cholinesterase inhibitor molecule.The four phenothiazine derived antipsychotic agent employed within this research all have shown potential to inhibit human recombinant cholinesterase enzymes. These phenothiazine compounds were all shown to possess selectivity towards BuChE. In addition, their BuChE inhibitory potential were found higher to the activity of the three drug molecules used as reference within the study. Although the phenothiazines employed have shown lower activity in comparison to galantamine, and donepezil in terms of AChE inhibition, it has been found better and comparable to the activity of rivastigmine. Thioridazine was found to be the most potent inhibitor among the phenothiazines employed.

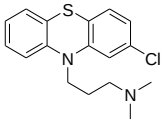
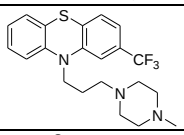
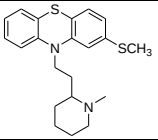
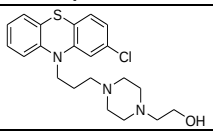
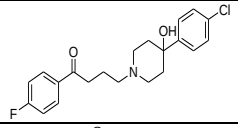
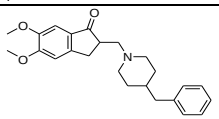
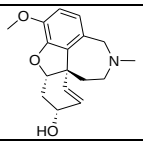
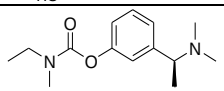
Haloperidol, a butyrophenone class antipsychotic agent, displayed quite different properties. First of all, haloperidol displayed selectivity towards AChE, rather than to BuChE seen for phenothiazines. Furthermore, its AChE inhibitory potential was lower than the potential of phenothiazines. It is noteworthy to mention that its BuChE inhibitory potential was the lowest one among the compounds tested.

The results definitely have shown that antipsychotic agents, depending on the class, have varying potentials to inhibit human cholinesterase enzymes. Both the phenothiazine class test compounds and the haloperidol have Aryl-spacer- tertiary amine pharmacophore, however; the organization of the pharmacophore is different in such a way that the spacer group in phenothiazines sources out from the center of the phenothiazine ring, while it is linked to the terminal of a relatively small aryl group in haloperidol. This aids in a T-shape Aryl-Spacer

organization in phenothiazine type BuChE inhibitors, while it is linear in haloperidol. This reveals out a bulkier aryl group organization in phenothiazines. In fact, it is known that the peripheral binding site of BuChE is larger in comparison the peripheral binging site of AChE (Rahman & Choudhary, 2014). Therefore, the organization of the Aryl group in phenothiazines does not fit the relatively smaller peripheral binding site of the AChE.

It is noteworthy to state that, although phenothiazines are cholinesterase inhibitors, they exert anticholinergic side effects (Sims, 1995). This is mainly attributed to their potential to bind to cholinergic receptors and to antagonize them (Sims, 1995) . However, considering the fact that the number of studies to find out original compounds designed to selectively inhibit BuChE, it is still important to question the chemical organization of phenothiazines yielding out BuChE selective cholinesterase inhibitor agents. Our results within this research have shown that phenothiazines can also inhibit human cholinesterase enzymes, selective to BuChE. Regarding the previous data obtained for the potential of phenothiazines to inhibit cholinesterase enzymes from various sources rather than human is in agreement with our findings.

Table 1: The potential of the test compounds to inhibit human recombinant cholinesterase enzymes

The test compound	Molecular structure	IC_{50} (µM) Human rec. AChE	IC_{50} (µM) Human rec. BuChE
Chlorpromazine		15.6 ± 0.2	3.4 ± 0.2
Trifluoperazine		9.8 ± 0.1	1.8 ± 0.2
Thioridazine		16.7 ± 0.6	0.6 ± 0.1
Perphenazine		11.6 ± 0.3	3.5 ± 0.1
Haloperidol		25.7 ± 1.4	144.6 ± 0.7
Donepezil		0.010 ± 0.0	9.6 ± 0.5
Galantamine		0.9 ± 0.1	12.6 ± 0.4
Rivastigmine		37.6 ± 0.3	15.9 ± 0.8

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