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Anticholinesterases

Zeynep Özdemir and Mehmet Abdullah Alagöz

Abstract

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are known serine hydrolase enzymes responsible for the hydrolysis of acetylcholine (ACh). Although the role of AChE in cholinergic transmission is well known, the role of BChE has not been elucidated sufficiently. The hydrolysis of acetylcholine in the synaptic healthy brain cells is mainly carried out by AChE; it is accepted that the contribution to the hydrolysis of BChE is very low, but both AChE and BChE are known to play an active role in neuronal development and cholinergic transmission. Myasthenia gravis (MG) is a muscle disease characterized by weakness in skeletal muscles and rapid fatigue. Anticholinesterases, which are not only related to the immune origin of the disease but also have only symptomatic benefit, have an indispensable role in the treatment of MG. Pyridostigmine, distigmine, neostigmine, and ambenonium are the standard anticholinesterase drugs used in the symptomatic treatment of MG. All of these compounds may increase the response of the myasthenic muscle to recurrent nerve impulses, primarily by protecting the endogenous ACh.

Keywords: acetylcholine, acetylcholinesterase, butyrylcholinesterase, anticholinesterases, neostigmine, pyridostigmine, distigmine, ambenonium, myasthenia gravis

1. Introduction

The autonomic nervous system (ANS) works out of our request, and it differs from the somatic system with this feature. Autonomic afferent and efferent fibers enter and exit the central nervous system through the spinal and cranial nerves. It connects with the medulla spinalis and intermediate neurons, which mediate autonomic reflexes in the brain stem [1, 2]. Changes in the internal and external environment and emotional factors affect autonomic activity through fibers, which descend from the hypothalamus. ANS shows its effect through neuromediators. Acetylcholine (ACh) and noradrenaline (NA) are the main neurotransmitters in the autonomic nervous system. ACh is released from all preganglionic endings. ACh is secreted from all postganglionic parasympathetic fibers, and it acts through muscarinic receptors [3, 4]. Autonomic nervous system disorders may occur with an abnormally high parasympathetic activity or abnormally low parasympathetic activity and/or abnormally high sympathetic activity or abnormally low sympathetic activity. MG, a neuromuscular junction disease that occurs due to ACh receptor deficiency in the postsynaptic membrane at the neuromuscular junction, is one of these disorders. The origin of the disease is thymus, because myoid cells form the source of receptor antigens. ACh release in the normal muscular junction leads to a localized end-plate potential, resulting in muscle contraction [5]. Although MG patients have normal nerve anatomy and function, there is a decrease in the number

of postsynaptic ACh receptors. During a muscle contraction under normal conditions, the release of ACh, which is caused by impulses along the axon, decreases gradually in each impulse. This decrease does not cause problems in the postsynaptic membrane when there is no pathology. However, in addition to the reduced number of receptors in MG, the ACh-receptor complex is also decreasing gradually; therefore, rapid fatigue is observed [6, 7].

2. Acetylcholine and cholinergic receptors

The neurotransmitter is ACh in all of the preganglionic autonomic fibers constituting the peripheral parts of the autonomic nervous system, in all postganglionic parasympathetic fibers and in several postganglionic sympathetic nerve fibers, and these ACh release fibers are called cholinergic fibers [8]. ACh is synthesized in the cytosol at the end of the nerve fibers, and then it is transported into the vesicles from the membrane of the vesicles (**Figure 1**). ACh is stored here in a very dense manner, with about 10,000 molecules in each vesicle. When an action potential reaches the nerve end, a large number of calcium channels are opened on the nerve end, since it has a large number of voltage-gated calcium channels.

Figure 1.
Biosynthesis, transmission, and inactivation of ACh [3].

As a result, the calcium concentration in the nerve end increases by 100 times; this increases the speed of incorporation of ACh vesicles with the nerve end membrane by 10,000 times. This incorporation allows the exocytosis of acetylcholine to the synaptic range by causing rupture of many vesicles. About 125 vesicles are usually ruptured with each action potential. The ACh is then broken up with AChE in a few milliseconds to the acetate ion and choline. Choline is taken back to the nerve end to be used in the formation of ACh again [9].

Studies have shown that many tissues respond to stimulation and inhibition are generated by compounds, which mimic the action of neuronal release of ACh or the neurotransmitter administered externally. Peripheral cholinergic receptors interacting with ACh are found in the parasympathetic postganglionic nerve endings in the smooth muscles and neuromuscular junction in the skeletal muscles. Cholinergic receptors are divided into two groups, namely nicotinic and muscarinic. The distribution of muscarinic receptors in the brain adapts to the distribution of ACh [10]. Receptors are mostly found in the striatum, neocortex, hippocampus, superior colliculus, locus coeruleus, and pons nuclei; whereas, the quantity of them in the hypothalamus, spinal cord, and cortex is low. These receptors in neuromuscular motor ends and ganglia are the first neurotransmitter receptors that have been isolated and purified in active form. It is contemplated that the receptor is comprised of two polypeptide chain monomers, which are connected to each other by a disulfide bond and have five subunits. When ACh is bound to these receptors, it allows the passage of the small cations such as Ca^{++} , Na^+ , and K^+ by leading to an increase in membrane permeability. The physiological effect of this condition is the formation of depolarization at the motor ends and consequently the muscular contraction or the continuation of nerve stimulation at the neuromuscular junction. Muscarinic receptors play an important role in regulating the functions of organs stimulated by the autonomic nervous system. The effect of ACh on these receptors in parasympathetic synapses may be stimulating or inhibiting. ACh both stimulates the secretion by activating the salivary glands and leads to the contraction of the respiratory system. The compound also inhibits cardiac contractions and relaxes the smooth muscles in the blood vessels. Recent studies have shown that there are five subtypes of muscarinic receptors (M_1 , M_2 , M_3 , M_4 , and M_5). M_1 is found in neuronal structures such as the central nervous system and ganglions, M_2 is found in the heart, M_3 is found in the smooth muscles in the glands, and M_4 is also found in the striatum and lungs [8–10].

2.1 Cholinesterase enzymes and cholinesterase inhibitors

AChE is a hydrolytic enzyme of the class of the serine hydrolase enzyme, which plays a major role in the hydrolysis of ACh in cholinergic synapses of the autonomic nervous system and central nervous system [11]. Electron microscopy studies using histochemical techniques have shown that this enzyme is located on both the nerve endings and the postjunction or postsynaptic membrane at the cholinergic synapses or junctions. This enzyme, which is also called main cholinesterase, hydrolyzes ACh most rapidly among choline esters. It can also hydrolyze methacholine, but it is ineffective against benzoylcholine. A second type of cholinesterase, which is called pseudocholinesterase, breaks down acetylcholine more slowly. Because this enzyme is the most rapidly broken choline ester butyrylcholine, pseudocholinesterase is called butyrylcholinesterase (BChE). Butyrylcholinesterase is not found in synapses and does not contribute to the hydrolysis of acetylcholine [12]. Both AChE and BChE are polymorphic and exist as homomeric and heteromeric molecular forms characterized by subunit relationships and hydrodynamic properties. Heteromeric molecular forms contain catalytic subunits linked to the lipid or triple helix collagen

tail and are often referred to as asymmetric or A forms of AChE. The G₁, G₂, and G₄ forms, which are homomeric hydrophilic globular forms of AChE, contain one, two, and four identical subunits, respectively. The G₄ form is secreted by neurons and secretory cells. An amphiphilic glycopospholipid-bound form is a dimer of G₂, which has two subunits having a glycopospholipid link to the cell membrane. Less polymorphism is observed in BChE, and only hydrophilic and asymmetric forms have been defined for this enzyme [11].

The structure of AChE reveals an active site containing a catalytic triad—glutamate (E327), histidine (H440), and serine (S200) at the base of a narrow valley about 20 Å depth. This general amino acid arrangement represents the serine hydrolase enzyme family. The gorge in AChE is coated with 14 aromatic amino acids consisting of phenylalanine, tyrosine or tryptophan, and the base of the gorge contains a number of anionic residues, which are collectively responsible for the interaction of ACh with the positively charged trimethylammonium group and the acceleration of binding of the cationic ligands. BChE contains six less aromatic amino acids than AChE in the gorge. These structural studies help to elucidate the molecular basis of the specificity between the active center and the ligand. In particular, the main substrate differences between AChE and BChE can be determined by the presence of two phenylalanines (F288 and F290), providing a rigid acyl binding pocket in AChE. In addition, replacement of these amino acids with leucine and valine to provide a less structurally restricted pocket in BChE can also determine the main substrate differences between AChE and BChE. In addition, AChE contains a peripheral anionic region responsible for allosteric inhibition by cationic ligand interactions in the catalytic region. This peripheral anionic region proposed by Changeux in 1966 links agents such as propidium to residues around the edge of the gorge. This peripheral anionic region may play a role in the catalytic process by mediating substrate inhibition [13–17].

The effects of ACh released from autonomic and somatic motor nerves are terminated by enzymatic degradation of the molecule by AChE that is present in high concentrations in cholinergic synapses and synthesized in both nerve and muscle tissues [18]. The drugs, which inhibit AChE, are called anticholinesterase agents. The characteristic pharmacological effects of anticholinesterases occur primarily by inhibiting the hydrolysis of ACh by the AChE enzyme in cholinergic pathways. Inhibition of cholinesterases induces ACh receptors by leading increased ACh in nerve synapses and neuromuscular junction. Continuous stimulation of ACh receptors results in cholinergic synaptic paralysis and central and peripheral clinical symptoms in the central nervous system, autonomic ganglia, parasympathetic and sympathetic nerve endings, and somatic nerves due to the accumulation of ACh in the motor end plates. Muscarinic effects due to parasympathetic activity and nicotinic effects due to sympathetic activity are seen. The main symptoms and signs depend on the balance between muscarinic and nicotinic receptors [8, 18].

Inhibition of the AChE prolongs the life of the neurotransmitter at the junction, thus resulting in pharmacological effects similar to those observed when acetylcholine is administered. AChE is the primary target of these drugs, but BChE is also inhibited. Anti-AChEs are used in the treatment of diseases such as myasthenia gravis, atony in the gastrointestinal tract, glaucoma, and Alzheimer's disease. These compounds are also used as nerve gases and insecticides. Anti-AChE agents can be divided into three groups based on their mechanism of action: competitive agonists, short-acting inhibitors, and long-acting inhibitors [19]. Before World War II, only reversible anti-ChE agents were known and their prototype is physostigmine. Organophosphates as highly toxic chemicals, which were first developed as agricultural insecticides, were also developed as a potential chemical warfare agent shortly before World War II. It is known that

the excessive toxicity of these compounds is due to the irreversible inactivation of AChE. Thus, organophosphate inhibitors are sometimes referred to as “irreversible cholinesterase inhibitors.” Strong nucleophiles such as pralidoxime can break phosphorus enzyme binding [9, 19].

Anti-AChEs, which are currently used for treatment in the postoperative period of intestinal system and atony of the smooth muscles of bladder, glaucoma, myasthenia gravis, and termination of the effects of competitive neuromuscular muscle relaxants produce nonselectively both muscarinic and nicotinic effects as indirect effects by increasing the ACh concentration. Long-acting and hydrophobic ChE inhibitors are also the only inhibitors with limited, well-documented efficacy in the treatment of dementia symptoms of Alzheimer’s disease [8, 20].

MG is a muscle disease characterized by weakness in skeletal muscles and rapid fatigue. Anti-AChEs, which are not only related to the immune origin of the disease but have only symptomatic benefit, have an indispensable role in the treatment of MG. Pyridostigmine, distigmin, neostigmine, and ambenonium are the standard anticholinesterase drugs used in the symptomatic treatment of MG. All of these compounds may increase the response of the myasthenic muscle to recurrent nerve impulses, primarily by protecting the endogenous ACh [8, 21, 22].

2.1.1 Pyridostigmine

The most commonly used anti-ChE in daily treatment is pyridostigmine bromide (**Figure 2**). The effect of the drug starts in 15–30 min, reaches maximum in 1–2 hours, and lasts 3–4 hours or longer. Pyridostigmine bromide, used for the treatment of MG and for protection against exposure to nerve agents, is a carbamate-derived reversible AChE inhibitor [23–25]. Due to the quaternary amine structure, it is relatively weakly absorbed from the gastrointestinal system. The elimination half-life of pyridostigmine bromide after a single dose of 60 mg in healthy volunteers was found to be 200 min. This requires frequent usage. Ninety-nine percent of AChE inhibitors, including pyridostigmine bromide, are administered orally [26–28].

2.1.2 Distigmine

Distigmine is a carbamate-derived reversible ChE inhibitor. The compound synthesized chemically by Schmid has a chemical structure consisting of two molecules of pyridostigmine bonded together by hexamethylene bonds (**Figure 3**). Distigmine is clinically used in some Asian and European countries, including Japan and Germany, and the main clinical indication for distigmine is myasthenia gravis.

Figure 2.
Structure of pyridostigmine bromide.

However, in Japan, distigmin was also used for glaucoma and underactive bladder [29, 30].

2.1.3 Neostigmine

Neostigmine (**Figure 4**) is commonly used to reverse nondepolarizing neuromuscular blocking agents. The drug increases the rate of recovery from moderate nondepolarizing neuromuscular blockade and reduces the incidence of residual blockade. However, doses of neostigmine used in clinical practice may cause muscle weakness when administered after complete recovery from neuromuscular blockade. Since the first studies investigating the effects of neostigmine were performed in anesthetized patients, the results may be mixed with the presence of anesthetic agents, which are known to be in the neuromuscular blockade. Later, the effect of neostigmine was supported by the studies that the volunteers did not receive anesthetic agents. However, the effects of neostigmine on maximum voluntary muscle strength have not been previously investigated [31, 32].

2.1.4 Ambenonium

In addition, compounds, which are structurally different from the above-mentioned carbamates for the treatment of MG, are also used. One of them, bisquaternary inhibitor ambenonium dichloride (**Figure 5**), is known to be one of the compounds with the highest inhibition ability against AChE in sub-nM range [33].

The superior effect potential is unique for the compound, which does not form any covalent bonds with the active site of the enzyme. Binding studies on the AChE-ambenonium complex have shown that the compound is capable of making very

Figure 3.
Structure of distigmine.

Figure 4.
Structure of neostigmine.

convenient contacts with the amino acids of the catalytic and peripheral AChE sites. Ambenonium produces less muscarinic side effects than carbamates. Unlike short-acting anti-AChE compounds, it is advantageous because it produces a larger and longer-lasting therapeutic effect during the night and waking period. In addition, the bisquaternary structure inhibits the passage from blood-brain barrier (BBB) after a conventional oral or intravenous route of administration. Another anti-AChE compound, edrophonium chloride (**Figure 6**), is used as a diagnostic tool for MG. It has a rapid onset and short pharmacological effect, so it cannot be used for therapeutic purposes [33, 34].

The optimal single oral dose of the anti-ChE agents can be determined empirically, when the MG is diagnosed. Basic records are made for a range of signs and symptoms, which reflect comprehension strength, vital capacity, and the strength of various muscle groups. The patient is given an oral dose of pyridostigmine at 30–60 mg, neostigmine at 7.5–15 mg, or ambenonium at 2.5–5 mg. Improvement in muscle strength and changes in other symptoms are recorded at frequent intervals until they return to the baseline state. After a baseline hour or longer, the drug is reintroduced, the dose is increased to one and a half times the initial amount, and the same observations are repeated. These repeats continue with increments of half the initial dose until the optimal dose is achieved. The duration of action between the oral doses is required to maintain the muscle strength of these drugs, which is usually 2–4 h for neostigmine, 3–6 h for pyridostigmine, or 3–8 h for ambenonium. However, the required dose may vary from day to day. Physical or emotional stress, intercurrent infections, and menstruation usually require an increase in the frequency or size of the dose. Unpredictable exacerbations and remissions of the myasthenic condition may require adjustment of the dosage. Pyridostigmine has sustained release tablets containing a total of 180 mg, 60 mg of this is released immediately and the drug concentration is 120 mg for several hours. This preparation is valuable in maintaining patients in periods of 6–8 h, but it should be limited to use before bedtime [5, 8, 35].

There is always a risk of cholinergic crisis, if the effects of anti-ChE drugs are weak and there is no any improvement in the symptoms of the disease even with high doses of AChE. In cholinergic crisis, nausea, vomiting, sweating, salivation, colic, diarrhea, miosis, bradycardia, etc., are observed and myasthenic weakness increases [7]. In addition, many drugs, including curariform agents,

Figure 5.
Structure of ambenonium.

Figure 6.
Structure of edrophonium.

certain antibiotics, and general anesthetics prevent neuromuscular transmission. Therefore, the application of these drugs to patients with MG requires an appropriate adjustment of the anti-ChE dose and other measures [8].

2.2 Newly developed cholinesterase inhibitors for MG

Muscarinic cardiovascular and gastrointestinal side effects of anti-ChE agents can usually be controlled by atropine or other anticholinergic drugs. However, these anticholinergic drugs mask many side effects of an excessive amount of anti-ChE agents. Tolerance in most patients may eventually lead to muscarinic effects [5, 7, 35, 36]. For these reasons, it is aimed to investigate more effective drugs for the treatment of MG and to prevent the hepatotoxicity and known gastrointestinal side effects, while creating the targeted pharmacological effect with the synthetic analogues at the development stage.

Musilek et al. performed the synthesis of 20 new bis-isoquinolinium inhibitors (**Figure 7**) in a study and determined whether the compounds would be effective in the treatment of MG. They evaluated the newly prepared compounds *in vitro* on human recombinant AChE and human plasmatic BChE and compared the inhibitory capabilities of the compounds expressed as IC_{50} with ambenonium dichloride, edrophonium chloride, BW284c51, and ethopropazine hydrochloride, which have been selected as standard. In three of the compounds they have obtained, they had promising results in which their compounds inhibited both enzymes better than or similar to edrophonium and BW284c51, however, worse than ambenonium *in vitro*. The kinetic assays are confirmed noncompetitive inhibition of human-recombinant AChE (hAChE) with two promising new compounds selected [37].

In a study in which neostigmine, pyridostigmine, and physostigmine quaternary phenylcarbamates were synthesized and evaluated their activity, N-monophenylcarbamate analogues together with their precursors of neostigmine methyl sulfate and pyridostigmine bromide and the N-methylammonium analogues of phenserine, tolserine, cymserine, and phenylethylcymserine were synthesized as long-acting peripheral inhibitors of AChE or BChE (**Figure 8**). According to the results of the study, only N-phenylcarbamate of 3-dimethylamiophenol to N-phenylcarbamate of 3-hydroxy-1-methylpyridinium bromide compounds had marginal ChE inhibitor of activity and compound N(1)-methylammonium bromide of (–)-phenserine, (–)-tolserine, (–)-cymserine, and (–)-phenylethylcymserine were strong anti-ChEs [38].

Monarsen (EN101) is an antisense oligodeoxynucleotide, which acts at the level of mRNA and selectively reduces the production of the enzymatic isoform of read-through AChE (AChE-R) by the destruction of the AChE-R mRNA. This compound selectively lowers the AChE-R levels in both blood and muscle, but, does not affect the synaptic variant of synaptic AChE (AChE-S). It was tested in experimental

Figure 7.
Structure of bis-isoquinolinium derivatives.

Figure 8.

Structure of neostigmine N-phenylcarbamate of 3-dimethylamino-phenol and 3-hydroxy-1-methylpyridinium and N(1)-methylammonium bromide of (-)-phenserine, (-)-tolserine, (-)-cymserine, and (-)-phenylethylcymserine.

autoimmune MG rats that oral or intravenous administration of EN101 reduced AChE in blood and muscle and increased survival, muscle strength, and disease severity. The stabilization of the reduction of the compound motor action potential (CMAP) on the responsive neurostimulation system and muscles was also observed during the entire treatment. This effect has been found to be comparable to that of pyridostigmine, which is worn out for hours and causes significant fluctuations in muscle strength. It was also found that clinical and electrophysiological improvement was associated with a decrease in autoimmune responses [39–41].

3. Conclusions

Anti-ChEs have an indispensable role in the symptomatic treatment of the MG, which is not directed against the immune origin. AChE inhibitors improve neuromuscular conduction by preventing disruption of circulating ACh in the neuromuscular junction. The compounds used in the treatment of MG have a positive charge in the molecule to provide the peripheral effect of the action and minimal blood-brain barrier penetration. However, the most prescribed carbamate inhibitors may cause many serious side effects, such as carbamylation of AChE. As a result, it is important to individually arrange treatment for each MG patient. The effect of treatment should be optimized for vital muscles such as respiratory and swallowing; because, different muscles are affected by varying levels. Since the nonselective AChE inhibitors are the most effective compounds at the beginning of MG, the dosage of AChE inhibitors is ideally reduced as they develop strength by immunosuppressive therapy. Decrease in activity over time may be related to increased levels of the AChE-R isoform, which may cause morphological and physiological abnormalities in the neuromuscular junction. Myasthenia gravis (MG) is usually caused by antibodies either to the acetylcholine receptor (AChR) or to the muscle-specific tyrosine kinase (MuSK) or at the neuromuscular junction. Patients with MuSK antibodies generally do not respond to the treatment; whereas, patients with AChR antibodies respond specifically to the treatment. For these reasons, EN101, a selective AChE inhibitor that specifically targets the isoform of AChE (AChE-R), has been developed recently and the AChR-antibody may be important for symptomatic relief in seropositive MG.

Conflict of interest

None to declare.

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References

- [1] Snell R. The Autonomic nervous system. In: Clinical Neuroanatomy. 7th ed. Philadelphia: Lippincott Publishers; 2010. pp. 120-125
- [2] Lantsova VB, Sepp EK, Kozlovsky AS. Role sympathetic autonomic nervous system in the regulation of immune response during myasthenia. *Bulletin of Experimental Biology and Medicine*. 2011;**151**(3):353-355. DOI: 10.1007/s10517-011-1328-6
- [3] Lemke T, Williams DA, Roche VF, Zito SW. Foye's Principles of Medicinal Chemistry. Philadelphia: Lippincott Williams & Wilkins; 2008. pp. 309-339
- [4] Akyüz G, Akdeniz-Leblebiciler M. Anatomy and assessment of the autonomic nervous system. *Turkish Journal of Physical Medicine and Rehabilitation*. 2012;**58**(Suppl 1):1-5
- [5] Mehndiratta MM, Pandey S, Kuntzer T. Acetylcholinesterase inhibitor treatment for myasthenia gravis. *Cochrane Database of Systematic Reviews*. 2011;**2**:CD006986. DOI: 10.1002/14651858.CD006986
- [6] Drachman DB. Myasthenia gravis; review article in medical progress. *The New England Journal of Medicine*. 1994;**330**(25):1797-1810. DOI: 10.1056/NEJM199406233302507
- [7] Meriggioli MN, Sheng JR, Li L, Prabhakar BS. Strategies for treating autoimmunity: Novel insights from experimental myasthenia gravis. *Annals of the New York Academy of Sciences*. 2008;**1132**:276-282. DOI: 10.1196/annals.1405.02
- [8] Goodman Gilman A, Hardman JG, Limbird LE. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill Medical Publishing Division; 2001. pp. 201
- [9] Guyton AC, Hall JE. Medical Physiology. 11th ed. Vol. 87-89. Philadelphia, Pennsylvania: Elsevier Saunders; 2000. p. 751
- [10] Zisimopoulou P, Lagoumintzis G, Poulas K, Tzartos SJ. Antigen-specific apheresis of human anti-acetylcholine receptor autoantibodies from myasthenia gravis patients' sera using *Escherichia coli*-expressed receptor domains. *Journal of Neuroimmunology*. 2008;**200**:133-141. DOI: 10.1016/j.jneuroim.2008.06.002
- [11] Colovic MB, Krstic DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. *Current Neuropharmacology*. 2013;**11**(3):315-335. DOI: 10.2174/1570159X11311030006
- [12] Chatonnet A, Lockridge O. Comparison of butyrylcholinesterase and acetylcholinesterase. *The Biochemical Journal*. 1989;**260**(3):625-634
- [13] Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, et al. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *Journal of Medicinal Chemistry*. 2012;**55**:10282-10286. DOI: 10.1021/jm300871x
- [14] Dvir H, Silman I, Harel M, Rosenberry TL, Sussmana JL. Acetylcholinesterase: From 3D structure to function. *Chemico-Biological Interactions*. 2010;**187**: 10-22. DOI: 10.1016/j.cbi.2010.01.042
- [15] Anand P, Singh P. A review on cholinesterase inhibitors for Alzheimer's disease. *Archives of Pharmacal Research*. 2013;**36**:375-399. DOI: 10.1007/s12272-013-0036-3

- [16] Bourne Y, Taylor P, Radić Z, Marchot P. Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site. *The EMBO Journal*. 2003;22:1-12
- [17] Changeux JP. Responses of acetylcholinesterase from *Torpedo marmorata* to salts and curarizing drugs. *Molecular Pharmacology*. 1966;2:369-392
- [18] Pappano AJ. Cholinceptor-activating & cholinesterase-inhibiting drugs. In: Katzung BG, Master SB, Trevor AJ, editors. *Basic & Clinical Pharmacology*. 12th ed. New York: McGraw-Hill Medical Publishing Division; 2012. p. 97
- [19] Triggle DJ, Mitchell JM, Filler R. The pharmacology of physostigmine. *CNS Drug Reviews*. 2006;4(2):87-136. DOI: 10.1111/j.1527-3458.1998.tb00059.x
- [20] Kelle İ. The effects of anticholinesterase drugs on bethanechol-induced contractile responses in rat ileum smooth muscle. *Dicle Medical Journal*. 2007;34(3):155-163
- [21] Brenner T, Hamra-Amitay Y, Evron T, Boneva N, Seidman S, Soreq H. The role of readthrough acetylcholinesterase in the pathophysiology of myasthenia gravis. *The FASEB Journal*. 2003;17:214-222
- [22] Sun C, Meng F, Li Y, Jin Q, Li H, Li F. Antigen-specific immunoadsorption of antiacetylcholine receptor antibodies from sera of patients with myasthenia gravis. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology*. 2010;38(2):99-102. DOI: 10.3109/10731191003634778
- [23] Strelnik AD, Petukhov AS, Zueva IV, Zobov VV, Petrov KA, Nikolsky EE, et al. Novel potent pyridoxine-based inhibitors of AChE and BChE, structural analogs of pyridostigmine, with improved in vivo safety profile. *Bioorganic & Medicinal Chemistry Letters*. 2016;26(16): 4092-4094. DOI: 10.1016/j.bmcl.2016.06.070
- [24] Amancio GCS, Grabe-Guimarães A, Haikel D, Moreau J, Barcellos NMS, Lacampagne A, et al. Effect of pyridostigmine on in vivo and in vitro respiratory muscle of mdx mice. *Respiratory Physiology & Neurobiology*. 2017;243:107-114. DOI: 10.1016/j.resp.2017.06.001
- [25] Machado-Alba JE, Calvo-Torres LF, Avira-Mendoza A, Mejia-Velez CA. Prescription profile of pyridostigmine use in a population of patients with myasthenia gravis. *Muscle & Nerve*. 2017;56:1041-1046. DOI: 10.1002/mus.25625
- [26] Lorke DE, Petroianu GA. Reversible cholinesterase inhibitors as pretreatment for exposure to organophosphates. A review. *Journal of Applied Toxicology*. 2018;1-16. DOI: 10.1002/jat.3662
- [27] Hegazy N, Demirel M, Yazan Y. Preparation and in vitro evaluation of pyridostigmine bromide microparticles. *International Journal of Pharmaceutics*. 2002;242:171-174
- [28] Petrov KA, Kharlamova AD, Lenina OA, Nurtdinov AR, Sitdykova ME, Ilyin VI, et al. Specific inhibition of acetylcholinesterase as an approach to decrease muscarinic side effects during myasthenia gravis treatment. *Scientific Reports*. 2018;8:304. DOI: 10.1038/s41598-017-18307-9
- [29] Schmid O. Bis-Carbamic Acid Ester Compounds, and A Process of Making Same. U.S. Patent 2789981;1957
- [30] Obara K, Chino D, Tanaka Y. Long-lasting inhibitory effects of distigmine on recombinant human acetylcholinesterase activity. *Biological & Pharmaceutical*

Bulletin. 2017;**40**:1739-1746. DOI: 10.1248/bpb.b17-00351

[31] Churchill-Davidson HC, Christie TH. The diagnosis of neuromuscular block in man. *British Journal of Anaesthesia*. 1959;**31**:290-301

[32] Kent NB, Liang SS, Phillips S, Smith NA, Khandkar C, Eikermann M, et al. Therapeutic doses of neostigmine, depolarising neuromuscular blockade and muscle weakness in awake volunteers: a double-blind, placebo-controlled, randomized volunteer study. *Anaesthesia*. 2018;**73**:1079-1089. DOI: 10.1111/anae.14386

[33] Komloov M, Musilek K, Horova A, Holas O, Dohnal V, Gunn-Moore F, et al. Preparation, *in vitro* screening and molecular modelling of symmetrical bis-quinolinium cholinesterase inhibitors—implications for early Myasthenia gravis treatment. *Bioorganic & Medicinal Chemistry Letters*. 2011;**21**:2505-2509. DOI: 10.1016/j.bmcl.2011.02.047

[34] Barber HE, Calvey TN, Muir KT, Taylor K. The effect of edrophonium on erythrocyte acetylcholinesterase and neuromuscular function in the rat. *British Journal of Clinical Pharmacology*. 2012;**56**(1):93-99

[35] Gold R, Schneider-Gold C. Current and future standards in treatment of myasthenia gravis. *Neurotherapeutics*. 2008;**5**(4):535-541

[36] Ra P, Stalberg E. Acetylcholinesterase inhibitors in myasthenia gravis: to be or not to be? *Muscle & Nerve*. 2009;**39**:724-728

[37] Musilek K, Komloova M, Holas O, Hrabínova M, Pohanka M, Dohnal V, et al. Preparation and *in vitro* screening of symmetrical bis-isoquinolinium cholinesterase inhibitors bearing various connecting linkage—Implications for early Myasthenia gravis

treatment. *European Journal of Medicinal Chemistry*. 2011;**46**(2):811-818

[38] Yu QS, Holloway HW, Luo W, Lahiri DK, Brossi A, Greig NH. Long-acting anticholinesterases for myasthenia gravis: Synthesis and activities of quaternary phenylcarbamates of neostigmine, pyridostigmine and physostigmine. *Bioorganic & Medicinal Chemistry*. 2010;**18**:4687-4693. DOI: 10.1016/j.bmc.2010.05.022

[39] Argov Z, McKee D, Agus S, Brawer S, Shlomowitz N, Yoseph OB, et al. Treatment of human myasthenia gravis with oral antisense suppression of acetylcholinesterase. *Neurology*. 2007;**69**:699-700. DOI: 10.1212/01.wnl.0000267884.39468.7a

[40] Brus B, Kosak U, Turk S, Pislár A, Coquelle N, Kos J, et al. Discovery, biological evaluation, and crystal structure of a novel nanomolar selective butyrylcholinesterase inhibitor. *Journal of Medicinal Chemistry*. 2014;**57**:8167-8179

[41] Angelini CI, Martignago S, Bisciglia M. New treatments for myasthenia: A focus on antisense oligonucleotides. *Drug Design, Development and Therapy*. 2013;**7**:13-17