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REVIEW ARTICLE

NATURAL CHOLINESTERASE INHIBITORS FROM MUSHROOMS

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Summary

Natural cholinesterase inhibitors were found in many biological sources: bacteria, blue-greens, plants, marine sponges, microscopic fungus, and in a smaller scale also in mushrooms, fruiting body of macroscopis fungus. Only cholinesterase inhibitors isolated from mushrooms are subjects of this minireview. These natural compounds with anticholinergic activity may be considered as prospective drugs against Alzheimer's disease.

Key words: natural cholinesterase inhibitor; mushroom; alkaloid; Alzheimer's disease

INTRODUCTION

Human body has two distinct enzyme cholinesterase activities: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE functions in the transmission of nerve impulses, whereas the physiological function of BuChE remains unknown. AChE is one of the crucial enzymes in the central and peripheral nerve system (Patočka et al., 2004).

Inhibition of cholinesterases, mainly AChE, and therefore prevention of acetylcholine degradation in synapses of cholinergic system is one of the most accepted palliative therapy opportunities for Alzheimer´s disease (AD) today (Birks, 2006). Since the introduction of the first cholinesterase inhibitor in 1997, most clinicians would consider the cholinergic drugs, donepezil (**I**) (Waldemar et al., 2011), rivastigmine (**II**) (Birks et al., 2009), and galantamine (**III**) (Prvulovic et al., 2010), to be the first line pharmacotherapy for mild and moderate AD. The most that these drugs could achieve is to modify the manifestations of AD. Due to a lack of selectivity of cholinesterase inhibitor drugs on the market, AD-patients suffer from side effects like nausea or vomiting.

Donepezil (**I**), centrally acting reversible AChE inhibitor is fully synthetic compound but rivastigmine (**II**), though also synthetic, is a derivate of natural carbamate cholinesterase inhibitor physostigmine (**IV**), alkaloid from West African shrub *Physostigma venenosum* (Zhao et al., 2004). Third cholinesterase inhibitor used at present in AD therapy is a natural alkaloid galanthamine (**III**) isolated from the Caucasian snow-drop (*Galanthus woronowii*) and from the bulbs of different species of the *Amaryllidaceae* family (Marco and Carmo Carreiras, 2006). Also the next cholinesterase inhibitor and perspective remedy of AD is a naturally occur-

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ring sesquiterpene alkaloid huperzine A (**V**) found in the Chinese firmoss *Huperzia serrata* (Desilets et al., 2009). All these drugs have highly different chemical structures and slightly different pharmacological properties (Figure 1), but they all work by inhibiting the breakdown of acetylcholine, an important neurotransmitter associated with memory, by blocking the enzyme AChE (Larner, 2010).

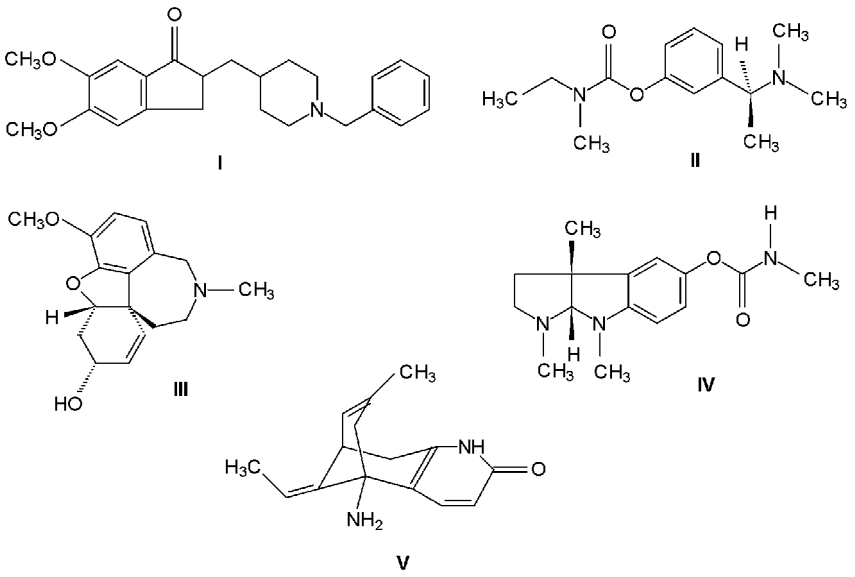

Figure 1. Chemical structures of cholinesterase inhibitors used in the treatment of Alzheimer's disease. I – Donepezil, II – Rivastigmine, III – Galanthamine, IV – Physostigmine, V – Huperzine A.

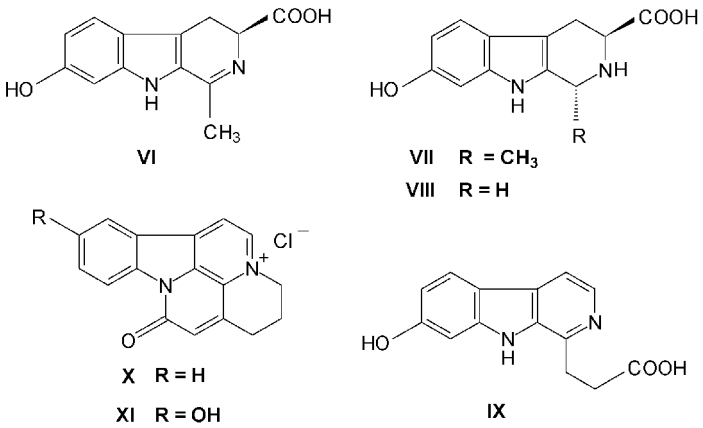

Figure 2. Chemical structures of cholinesterase inhibitors isolated from mushrooms.
VI – Brunnein A, VII – Brunnein B, VIII – Brunnein C, IX - 3-(7-hydroxy-9H-beta-carboline-1-yl)propanoic acid, X – Infractopicrin, IX – 10-Hydroxy-infractopicrin.

CHOLINESTERASE INHIBITORS IN MUSHROOMS

Interesting natural cholinesterase inhibitors were also found in some mushrooms (Figure 2). Four beta-carboline alkaloids, brunneins A-C (**VI-VIII**) and 3-(7-hydroxy-9H-beta-carboline-1-yl)propanoic acid (**IX**), were found in fruiting bodies of the agaricoid fungus *Cortinarius brunneus*[1] . Brunnein A (**VI**) exhibited very low cholinesterase inhibitory effects and no cytotoxicity (Teichert et al., 2007). From other mushroom family *Cortinariaceae*,

Cortinarius infractus Berk., two alkaloids, infractopicrin (**X**) and 10-hydroxy-infractopicrin (**XI**), were isolated by Norbert Arnold and co-workers from Leibniz Institute of Plant Biochemistry (Geissler et al., 2010). Both compounds show AChE-inhibiting activity and possess a higher selectivity than galanthamine. Docking studies show that lacking π-π-interactions in BuChE are responsible for selectivity. Studies on other AD pathology related targets show an inhibitory effect of both compounds on self-aggregation of Abeta-peptides but not on a AChE induced Abeta-peptide aggregation. Low cytotoxicity as well as calculated pharmacokinetic data suggest that the natural products could be useful candidates for further drug development.

Another natural anti-cholinesterases of unknown chemical structure have been observed in mushrooms of family *Agaricus*. The ethyl acetate and hexane extract of *Agaricus bitorquis*[2] and the hexane extract of *Agaricus essettei*[3] showed meaningful anti-BuChE activity being close to that of galantamine (Öztürk et al., 2011).

ANTICHOLINESTERASE ACTIVITY AND CHOLINESTERASE SELECTIVITY

Molecular topography of both active sites of AChE and BuChE is different and different are also affinities of inhibitors to them (Patočka et al., 2004). Affinities of cholinesterase inhibitors discussed in this paper are summarized in Table I. The affinity is expressed as IC_{50} value. i.e. half of the maximum

Table 1. Affinity and selectivity of some cholinesterase inhibitors used as Alzheimer's disease remedy and some isolated from mushrooms

Compound	IC_{50} AChE (μM)	IC_{50} BuChE (μM)	Selectivity IC_{50}BuChE/IC_{50}AChE	References
I	0.323±0.126 0.033±0.012 *	12.85±0.70 -	39.8	Rakonczay, 2003 Snape et al., 1999
II	4.76±0.10	0.238±0.02	0.05	Rakonczay, 2003
III	8.70±0.05 5.00±0.17 0.35 **	24.4±2.84 59.2±1.70 18.6 ***	2.80 11.8 53.1	Birks, 2006 Rakonczay, 2003 Thomsen et al., 1990
IV	2.58±0.03 0.061±0.018 ****	1.34±0.28 0.014±0.006	0.519 0.230	Yu et al., 1988
V	0.064±0.012	53.6±8.7	837.5	Liu et al., 1998
VI	Very low	-	-	Teichert et al., 2007
VII	Very low	-	-	Teichert et al., 2007
VIII	Very low	-	-	Teichert et al., 2007
IX	Very low	-	-	Teichert et al., 2007
X	9.72±0.19	No inhibition	> 10	Geissler et al., 2010
XI	12.7±0.16	No inhibition	> 10	Geissler et al., 2010

Inhibition of cholinesterase activities was studied in a human brain cortex for AChE and in human sera for BuChE.
IC50 values (μM) were determined and are reported as the means ± S.E. of 3–5 determinations.
* Rat brain AChE, ** Human erythrocyte AChE, *** Human plasma BuChE, **** Electric eel AChE

[1] *Cortinarius* is the biggest genus of mushrooms. Apart from a few species such as *C. caperatus*, many even so-called edible species appear to have very similar species which are at least inedible if not poisonous.
[2] *Agaricus bitorquis* is an edible white mushroom of the genus *Agaricus*, similar to the common button mushroom that is sold commercially.
[3] *Agaricus essettei* is an excellent edible mushroom

inhibitory concentration. The IC_{50} of inhibitors has been determined by constructing dose-response curves. The IC_{50} terminology is used to express anticholinesterase activity of cholinesterase inhibitors. The selectivity of cholinesterase inhibitors for BuChE versus AChE is also summarized in an attached table. However, it is not known if all tested inhibitors bind exactly to the same active site.

DISCUSSION

The selectivity of cholinesterase inhibitors for BuChE versus AChE, and for different molecular forms of AChE, may have an influence on both therapeutic and adverse effects. Donepezil and galanthamine are strong selective inhibitors of AChE, while the other agents also inhibit BuChE. The adverse effects associated with cholinergic hyperactivity are not due to blockage of BuChE as previously suggested. This is because they are seen with the AChE-selective inhibitors and not with those inhibitors that only inhibit BuChE. The advantage of nonselective inhibitors is that they may also increase acetylcholine levels by inhibiting BuChE in glial cells (Weinstock, 1999).

It is not yet known what cholinesterase inhibitors are better in AD treatment: selective inhibitors of AChE or selective inhibitors of BuChE? In human brains, BuChE is found in neurons and glial cells as well as in neuritic plaques and tangles in patients with AD. And although its physiological function is unknown, it may be important in AD ethiology (Giacobini, 2001). Some structure−activity studies predicted that compounds which are highly potent and selective inhibitors of human BuChE are useful in the treatment of AD (Furukawa-Hibi et al., 2011). Cholinesterase inhibitors are the primary treatment for the cognitive symptoms of AD, but the search for new and better cholinesterase inhibitors for clinical use is not finished. Cholinesterase inhibitors from mushrooms are only prospective drugs against Alzheimer's disease and it is illusory to estimate their clinical potential.

CONCLUSIONS

Cholinesterase inhibitors prevent reduction of acetylcholine via inhibiting AChE enzyme which hydrolyzes acetylcholine in the neuronal end from which it is released. The currently most accepted therapy of AD is the application of mild and reversible AChE inhibitors to restore acetylcholine levels and therefore cholinergic brain activity. These inhibitors play an important role in the treatment of AD. Some of them are natural bioactive compounds, secondary metabolites isolated from various plants. Most of these metabolites are not found outside of the fungal kingdom, therefore fungi constitute a valuable complementary source for novel lead compounds (Orhan and Sener, 2003; Rodrigues et al., 2005; Houghton et al., 2006).

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