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REVIEW

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Cotinine: A Potential New Therapeutic Agent against Alzheimer's disease

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SUMMARY

Tobacco smoking has been correlated with a lower incidence of Alzheimer's disease (AD). This negative correlation has been attributed to nicotine's properties. However, the undesired side-effects of nicotine and the absence of clear evidence of positive effects of this drug on the cognitive abilities of AD patients have decreased the enthusiasm for its therapeutic use. In this review, we discuss evidence showing that cotinine, the main metabolite of nicotine, has many of the beneficial effects but none of the negative side-effects of its precursor. Cotinine has been shown to be neuroprotective, to improve memory in primates as well as to prevent memory loss, and to lower amyloid-beta (Aβ)) burden in AD mice. In AD, cotinine's positive effect on memory is associated with the inhibition of Aβ aggregation, the stimulation of pro-survival factors such as Akt, and the inhibition of pro-apoptotic factors such as glycogen synthase kinase 3 beta (GSK3β). Because stimulation of the α7 nicotinic acetylcholine receptors (α7nAChRs) positively modulates these factors and memory, the involvement of these receptors in cotinine's effects are discussed. Because of its beneficial effects on brain function, good safety profile, and nonaddictive properties, cotinine may represent a new therapeutic agent against AD.

Alzheimer's disease: The Cholinergic System as a Therapeutic Target

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia [1, 2]. The disease is characterized by extracellular accumulation of senile plaques, mainly composed of aggregated forms of the amyloid-beta peptide (Aβ), as well as intracellular accumulation of neurofibrillary tangles of the microtubule-associated protein tau [1]. These neuropathological changes are associated with structural brain abnormalities, inflammation, and cognitive impairment such as impairment of working memory in AD patients. The progressive loss of memory in AD patients correlates with increased levels of Aβ and the deterioration of the cholinergic system in the brain. The degenerative process involves a sequence of pathological events, including early degeneration of the cerebral basal forebrain and subsequent deterioration of the cortical cholinergic system [3, 4]. This deterioration commonly includes a reduction in the levels of acetylcholine (ACh) and α3, α4, and α7 nicotinic ACh receptors (nAChRs) as well as a decrease in the activity of choline acetyltranferase in the brain [5].

Of these nAChRs, the α7 receptors are considered to be ideal therapeutic targets for several neurological conditions, including AD, schizophrenia, and Parkinson's disease (PD) as well as tobacco addiction [6, 7]. The α7nAChR is a homomeric pentamer that has a high permeability to calcium (P_{Ca}:$P_{Na} \approx 10$), and undergoes rapid and reversible desensitization and pronounced inward rectification [8]. The α7 subunit is highly expressed in the cortex, hippocampus, and hypothalamus [8], and has also been suggested to have functionally important expression in nonneuronal tissues such as cells of the immune system [9]. There is evidence suggesting that Aβ, which accumulates in the brain of AD patients, has a high affinity for the α7 receptors [10], acting as both an agonist [11] and an antagonist [12] at these receptors. Based on this evidence, it has been proposed that positive modulators of these receptors may be neuroprotective against Aβ toxicity and stimulate learning and memory [13].

Current therapies for AD improve the function of the cholinergic system; acetylcholinesterase inhibitors reduce the clearance and increase the availability of acetylcholine in the brain [14–16]. Another current therapeutic approach aims to decrease glutamate excitotoxicity by blocking the N-methyl D-aspartate (NMDA) glutamate receptor using the receptor antagonist memantine. Unfortunately, these therapies only slightly ameliorate cognitive deficits in AD patients and show only short-term effectiveness [17–21].

A negative correlation between tobacco use and the incidence of AD has been reported [22]. In research into components of tobacco that may enhance cholinergic function, nicotine

(3-[1-methyl-2-pyrrolidinyl] pyridine), an alkaloid derived from tobacco, has been extensively investigated. Nicotine binds to Aβ, blocking its aggregation into fibrils and is thereby neuroprotective [23]. Nicotine also diminishes AD pathology in animal models of the disease [24, 25]. However, clinical studies that aimed to determine the efficacy of nicotine against AD pathology have not shown a significant effect of nicotine in enhancing memory [26, 27] but rather a clear positive effect on attention in AD [27] and PD patients [25, 28]. The positive effect of nicotine on attention has been mostly attributed to its agonistic stimulation of the nAChRs, which plays an important role in mediating memory and attention processes [29–31]. It is likely that nicotine's effect on cognitive abilities may be counteracted by the nicotine-induced desensitization of the receptor. However, the failure of nicotine to improve memory in AD patients, its inherent toxicity, and the fact that it induces tachyphylaxis and addiction have discouraged its use in the clinical arena [32].

New evidence suggests that cotinine ([5S]-1-methyl-5-[3-pyridyl]-pyrrolidin-2-one), the main metabolite of nicotine, has similar beneficial properties against AD pathology as nicotine but does not have the adverse side-effects of nicotine. Specifically, it has been shown that cotinine prevented working and reference memory loss in a mouse model of AD (Tg6799) and prevented Aβ aggregation *in vitro* as well as plaque deposition *in vivo* [33].

Based on this evidence, this review discusses the potential of cotinine as an agent to prevent or treat AD.

Cotinine

In humans, more than 80% of nicotine is metabolized to cotinine by cytochrome P450 2A6 (CYP2A6) [34] and cytochrome P450 2A5 (CYP2A5) [35] enzymes [36]. The physiologically active form of cotinine, the (-)-isomer, accumulates in the body as a result of tobacco exposure. The metabolic rate of cotinine synthesis is determined by one's genetic background. For instance, it has been shown that individuals expressing a shorter form of CYP2A6 (i.e., CYP2A6*4) produce lower levels of cotinine [37]. The expression of different CYP2A6 variants may explain differences in the metabolism of cotinine in people of different ethnicities [38]. For example, CYP2A forms with low-enzymatic activity are represented differently in different ethnic groups, as observed in about 9.1% in white and 21.9% in black populations [38]. Cotinine, is mostly metabolized by the liver to its major metabolites, trans-3'-hydroxycotinine and its glucuronide [39–41]. Ethnicity affects the clearance of cotinine, with African Americans showing a lower average clearance of cotinine than Caucasians [42]. In addition to genetic factors, food consumption can also influence cotinine production [38], as grapefruit juice has been shown to inhibit the activity of CYP2A6 [43, 44].

Moreover, cotinine crosses the blood–brain barrier [45, 46] and is almost completely absorbed when administered orally. Despite its structural similarities with nicotine (Figure 1), cotinine has distinct pharmacological properties. Cotinine is 100 times less toxic than nicotine, has a longer half-life (20–24 h vs. 2 h, respectively), and is not addictive in humans [47].

The first studies showing some behavioral effects were performed in different species of animals. A seminal study performed by Yamamoto et al., showed that intravenously administered cotinine slightly increased in EEG activity and behavioral arousal in cats with a minor decrease in blood pressure [48]. In another study, Risner et al. [49] reported the behavioral effects of nicotine compared with those of its metabolites, nornicotine and cotinine, in beagle dogs and squirrel monkeys. Subjects responded under a multiple fixed-interval (FI) 300-seconds, fixed-ratio (FR) 30 response schedule of food presentation. In the dogs, cotinine (0.01–10.0 mg/kg i.m.) produced only dose-dependent decreases in rates of responding during both FI and FR components. In the squirrel monkeys, however, cotinine (0.1–3.0 mg/kg i.m.) increased responding during FI components; a high dose of 30.0 mg/kg decreased responding during both FI and FR components. These studies initially suggested that cotinine may have behavioral effects. More recently, we and other laboratories have found that cotinine is a memory enhancer in various animal models of disease. For example, cotinine improved the working memory performance of adult rhesus monkeys (*Macaca mulatta*) in the delayed matching-to-sample task (DMTS). Also, we recently published evidence showing that cotinine (5 mg/kg) decreased anxiety and enhanced the extinction of contextual fear memory after fear conditioning in wild type mice [50]. In addition, in the transgenic (Tg) mouse model of AD (Tg6799), long-term treatment with cotinine prevented working and reference memory impairment, as tested in three different learning and memory tasks, including circular platform, cognitive interference, and radial arm water maze tests. The doses effective in the Tg6799 mice were within the same range as those that improved working memory in primates [51, 52]. In addition to its mnemonic qualities, cotinine has anti-Aβ aggregation properties that add to its value as a new treatment for AD [33].

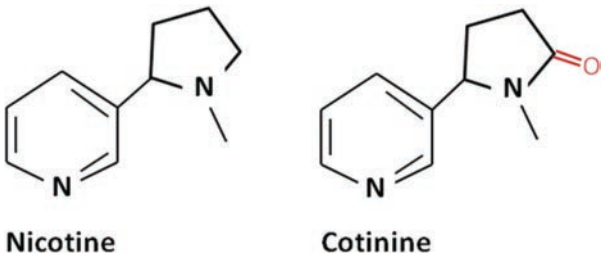

Figure 1 Comparison of the structures of cotinine and nicotine.

The pro-cognitive effect of cotinine in the Tg6799 mice may be explained by the reduction in the level of the aggregated forms of Aβ, including Aβ plaques and oligomeric forms of the peptide, in the hippocampus and cortex [33]. An advantage of cotinine is that it is not only an anti-Aβ aggregation compound but also a molecule that stimulates signaling pathways that support memory and brain homeostasis. For instance, cotinine induced the activation of the pro-survival protein kinase B (Akt)/glycogen synthase kinase 3β (GSK3β) pathway in the brains of both Tg6799 and wild type control mice, suggesting that the activation of these factors is independent from its effect on Aβ aggregation [33]. Interestingly, the Akt/GSK3β pathway is stimulated by the α7 receptor, has roles inhibiting neuronal cell death, and is involved in promoting neuronal synaptic plasticity and long-term potentiation

[53–55]. Therefore, $\alpha 7$ receptors and the Akt/GSK3β pathway are considered therapeutic targets for improving memory and attention in individuals with various neurological disorders, including AD [13, 56].

Nicotinic Acetylcholine Receptors (nAChRs) as a Therapeutic Target against Alzheimer's disease

Nicotinic acetylcholine receptors are cationic, ligand-gated channels that mediate fast neurotransmission in the central and peripheral nervous systems [57]. The $\alpha 7$ and $\alpha 4\beta 2$ nAChR subtypes are the most abundant of the nicotinic receptors and are fundamental for mediating working memory and attention in mammals. These receptors are localized throughout the cortex, hippocampus, amygdala, hypothalamus, striatum, and other regions involved in these cognitive processes [13]. Because decreased levels of these receptors have been found in AD brains [58], this reduction is considered to explain, at least in part, the cognitive deficits in AD.

The $\alpha 7$ receptors are very important for mediating sensory gating, attention and learning, and memory, making them an ideal target to improve these cognitive functions. However, these receptors are susceptible to agonist-induced desensitization, a characteristic that complicates the use of agonists as therapeutic agents. The desensitization can also explain why many acetylcholinesterase inhibitors, which increase the synaptic levels of ACh, as well as drugs acting as agonists of the $\alpha 7$ receptors only induce modest and short-term therapeutic effects [59].

An alternative approach to treating memory and attention deficits that does not cause the tachyphylaxis induced by $\alpha 7$ nAChR agonists is the use of nonagonist positive modulators of these receptors [60], including partial agonists such as S24795 and GTS-21[61] or positive allosteric modulators (PAMs) [62] such as PNU-120596 [63, 64] and galantamine [65, 66]. For example, PAMs can facilitate ACh neurotransmission by binding to receptor regions other than the active site, changing the receptor conformation, and in some cases preventing agonist-induced desensitization [13].

In an attempt to improve the cognitive abilities of AD patients with this new approach, modulators of the $\alpha 7$ receptors, such as 3-(2,4-dimethoxybenzylidene) anabaseine (GTS-21 or DMXB-A) [56, 67, 68], and many others, are under development or are currently being investigated in clinical trials [56, 69–71].

Cotinine as a Modulator of the Nicotinic Acetylcholine Receptors

Cotinine is weak agonist of the $\alpha 7$ receptor, and whether this receptor is the main target of cotinine is still controversial [72]. However, the possibility that cotinine is an allosteric modulator of this receptor needs to be further explored.

Alternatively, to explain the beneficial effects of cotinine on cognitive abilities, it has been postulated that cotinine desensitizes nAChRs located on inhibitory GABAergic neurons of the hippocampus, provoking the activation of the excitatory glutamate receptors in this region of the brain, and thereby stimulating cognitive abilities [73, 74]. This hypothesis is interesting; however, direct evidence that cotinine desensitizes the hippocampal $\alpha 7$ receptor is still needed. Contrary to this idea, new evidence shows that chronic treatment with cotinine stimulates the Akt/GSK3β pathway in the hippocampus and cortex of AD and control littermate mice [33]. Because the Akt/GSK3β pathway is located downstream of the $\alpha 7$ nAChR, it is unlikely that desensitization of the $\alpha 7$ nAChR, which is highly expressed in these brain regions, could lead to the marked activation of these signaling pathway. It is feasible that instead of desensitizing $\alpha 7$ nAChRs, cotinine may act as a PAM of the human $\alpha 7$ nAChR. The positive modulation of these receptors would explain the positive effects of cotinine not only on learning and memory but also in reversing apomorphine-induced deficits in prepulse inhibition of acoustic startle in rats [75], a process modulated by the $\alpha 7$ nAChR.

Positive modulation of the $\alpha 7$ nAChRs could also explain cotinine's positive effect on neuronal survival because the activation of Akt stimulates pro-survival proteins (e.g., Bcl-2 and the transcription factor cAMP responsive element-binding protein [CREB; Ref. 11] and inhibits the pro-apoptotic protein c-Jun N-terminal kinase (JNK) via the activation of the apoptosis signal-regulating kinase (Ask) [76, 77; Figure 2). Furthermore, $\alpha 7$ nAChRs can favor synaptic plasticity and cognition by activating the protein kinases phosphoinositide-3 kinase [PI3K; Refs. 78, 79], Akt, extracellular signal-regulated kinase 1/2 (ERK1/2), and the transcription factor CREB, which participate in mediating the structural and molecular changes required for learning and memory processes [80, 81].

The activation of PI3K heterodimers p85/p110 by the $\alpha 7$ receptor is triggered by the binding of p85 (the regulatory subunit) to phospho-tyrosyl proteins such as Fyn, which leads to the release of p110 [the catalytic subunit; Ref. 82]. Thus, PI3K stimulates the phosphorylation of Akt at residues threonine 308 and serine 473 [83]. The active form of Akt can then promote neuronal survival by stimulating CREB and Bcl-2 activity and by inactivating Ask1 by phosphorylation at serine 83 [84].

Of equal importance, because GSK3β is considered to be one of the main tau kinases *in vivo,* the inhibition of GSK3β by cotinine may also prevent the abnormal phosphorylation of tau observed in AD brains and the consequent appearance of neurofibrillary tangles of hyperphosphorylated tau [85–87]. The potential inhibition of tau phosphorylation by cotinine is currently being investigated in our laboratory as a new target mechanism against AD.

Furthermore, the stimulation of both CREB and ERK1/2 by cotinine may promote the expression of activity-regulated cytoskeleton-associated protein (Arc, also termed Arg3.1), derived from an immediate early gene required for the consolidation of memory [88–90]. Arc expression is stimulated by brain-derived neurotrophic factor (BDNF), serum response element [91], NMDA receptors [92], elongation factor 2 [93], CREB, and ERK1/2 [94]. This versatile protein is believed to mediate memory storage in the brain's active networks by coupling changes in neuronal activity patterns to diverse forms of synaptic plasticity [95]. The $\alpha 7$ receptors seem to have a clear role in mediating Arc gene expression, as an increase in levels of Arc mRNA were found in rats treated with the selective $\alpha 7$ receptor partial agonist SSR180711 [96]. Thus, it is through the modulation of the $\alpha 7$ nAChRs and

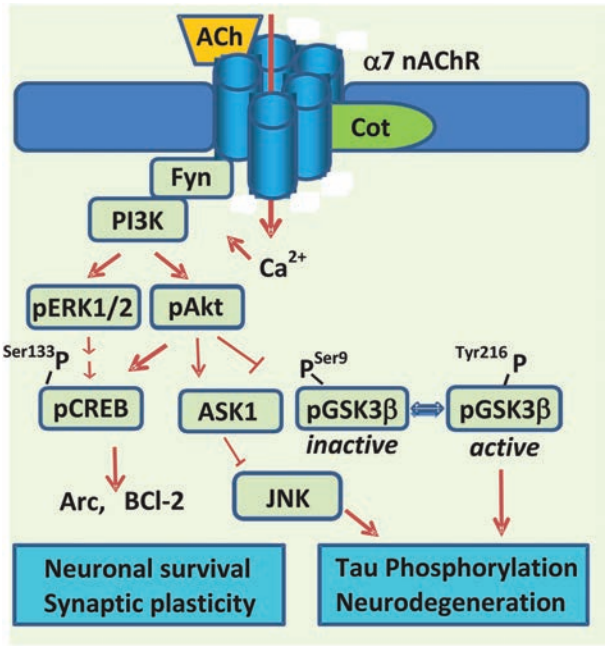

Figure 2 Hypothetical model of cotinine's potentiation of the α7 nAChR. The scheme represents the hypothetical positive allosteric modulation of the α7 nAChR by cotinine, and the activation of signaling pathways that are downstream of the a7 nAChR. The consequences of the activation by cotinine of components of the PI3K-Akt-GSK3β pathway on AD pathology are suggested. The activation of Akt by cotinine may result in the inhibition of the tau kinase GSK3β and consequently the formation of hyperphosphorylated forms of tau found in the neurofibrillary tangles (one of the neuropathological hallmarks of AD). Also, the inhibition of GSK3β may inhibit its pro-apoptotic effect. On the other hand, the stimulation of pERK can stimulate pCREB activity and as a result stimulate the expression of Arc, a protein that participate in the remodeling of the synapses during learning and memory processes and is required for long-term memory. CREB stimulate the expression of antiapoptotic factors such as Bcl2.

its associated pathways that cotinine may exhibit its pro-cognitive actions, giving rise to further potential implications in other pathologies that involve a downregulation of these cell signaling cascades.

Cotinine-inhibited Aβ_{1-42} Aggregation

According to the updated amyloid cascade hypothesis, AD is predominantly caused by the neurotoxicity of aggregated forms of soluble Aβ [97–100]. This concept, though still controversial, is supported by the fact that the level of soluble Aβ correlates better with dementia than does plaque burden in AD patients [101, 102].

In aqueous solutions, Aβ undergoes a time- and concentration-dependent transition from a soluble α-helical to an insoluble β-sheet structure. Aβ can exist as nontoxic soluble monomers, neurotoxic oligomers and protofibrils, or as insoluble fibrils. Because only the aggregated forms of Aβ are toxic, a great deal of translational research effort has focused on investigating compounds with anti-Aβ aggregation activity. One recent 78-week randomized, double-blind, placebo-controlled phase 2 study investigated ELND005 (an oral amyloid anti-aggregation agent) in mild to moderate AD. Therein, the anti-aggregation approach did not show significant clinical efficacy [103]. It is possible that the small sample size (n = 166 participants) masked a beneficial effect of this drug; however, it is likely that anti-aggregation drugs may need to target additional aspects of the pathology to achieve clinical efficacy. Studies with larger sample sizes and employing different anti-aggregation drugs are needed.

It has been postulated that the oligomerization and fibrillation processes are pathways that can occur sequentially or independently of each other. Thus, each of these pathways can be targeted separately or simultaneously by anti-aggregation compounds [104]. For example, some compounds such as curcumin inhibit oligomerization but not fibrillation [105], and other compounds such as *o*-vanillin inhibit the formation of both oligomers and fibrils [106]. Other molecules such as the naphthalene sulfonates inhibit fibrillation but not oligomerization [107]. Few compounds inhibit both processes, and it is these that may be the more promising agents for stopping Aβ toxicity *in vivo* [108, 109].

We and others have previously demonstrated that cotinine can inhibit both Aβ oligomerization and fibrillation [33]. Seminal studies have shown that cotinine binds to Aβ with high affinity (K_a = 0.1 nM) [110] and inhibits its fibrillation *in vitro* [23]. Recently, an atomic force microscopy analysis of Aβ_{1-42} fibrillation under conditions that favor fibrillation, such as high temperature (37°C) and high concentrations of the peptide (millimolar range), confirmed that cotinine inhibits Aβ_{1-42} aggregation *in vitro*, decreasing the average number and length of fibrils [33]. Further analysis of the effect of cotinine on Aβ_{1-42} oligomerization using dot blot techniques showed that cotinine inhibited Aβ_{1-42} oligomerization *in vitro* [111]. Consistent with a reduction in Aβ toxicity by inhibiting the aggregation of the peptide into the toxic species, we found that cotinine protected cultured cortical neurons against Aβ_{1-42} toxicity [111]. This anti-aggregation effect seems to underlie the reduction in Aβ oligomers and plaques observed in the brains of AD Tg6799 mice treated with cotinine. This decrease paralleled an overall improvement of reference and working memories in the Tg AD mice [112].

The interaction of cotinine with the full-length Aβ_{1-42} monomer at the atomic level was elucidated using molecular docking and molecular dynamics (MD) simulations. After the simulation, analysis of the most representative structure indicated that cotinine may interact with the histidine (His) 6, tyrosine (Tyr) 10, and His14 residues of Aβ_{1-42}. The interaction of cotinine with His6, Tyr10, and His14 residues of Aβ_{1-42} induces important structural changes in the peptide that seem to play a key role in reducing its aggregation. This analysis also indicated that the interaction with cotinine greatly influences the phenylalanine 20 to methionine 35 region of the full-length Aβ_{1-42} monomer [112]. This segment contains the loop (amino acids 24–28) and second hydrophobic domain (amino acids 29–35) both of which regions have been implicated in the aggregation process [113]. These results suggest that the interaction of cotinine with key residues in Aβ_{1-42} may induce critical changes in its secondary structure, inhibiting its aggregation.

Feasibility of using Cotinine as a Pharmacological Therapy against AD

Clinical studies assessing the effect of cotinine on the progression of AD have not yet been performed. However, the pharmacokinetic profiles and safety of orally and intravenously administered cotinine have been previously investigated in humans [47, 114–119]. A seminal study indicated that doses of up to 1800 mg of cotinine per day during a 4-day period were well tolerated in humans [114]. Later, another study investigated the safety and efficacy of several oral doses of cotinine fumarate, up to 160 mg per day during a period of 10 days, as an aid for tobacco cessation in abstinent cigarette smokers [47]. At the doses tested, cotinine did not show efficacy in reducing tobacco consumption but did show that, unlike nicotine, its metabolite did not elicit withdrawal effects, addictive behavior, or any negative cardiovascular effects, such as increasing heart rate or systolic or diastolic blood pressures [47]. The multiple mechanisms of action of cotinine and its good safety profile in humans make this drug an ideal candidate for preventing, delaying, or halting AD pathology.

Conclusions

AD is characterized by deterioration of the cholinergic system, including loss of cholinergic neurons and downregulation of nAChRs in the brain [120]. Nicotine itself shows limited use in the clinic because of its toxicity and addictive properties. However, cotinine prevents the memory loss and inhibits the amyloid burden in the brain in a mouse model of AD. Cotinine also inhibits the aggregation of Aβ peptides (considered the main cause of the pathology), activates the pro-survival enzyme Akt, and inhibits the pro-apoptotic factor GSK3β *in vivo*.

Conveniently, cotinine has almost a 10-fold longer half-life than nicotine and a good safety profile in humans. Cotinine has shown memory enhancing properties not only in mice, but in monkeys as well, indicating that its beneficial actions are not restricted to rodents. The new information about the effects of cotinine in the brain gives us a better understanding of cotinine's potential for the treatment of AD and opens a new avenue in the search for therapies for this devastating disease.

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Conflicts of Interest

The authors declare no conflict of interest.

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