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Is anti-cholinesterase therapy of Alzheimer's disease delaying progression?

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ABSTRACT. During the last decade, a systematic effort to develop a pharmacological treatment for Alzheimer's disease (AD) resulted in three drugs being registered for the first time in the US and Europe. All three compounds are cholinesterase inhibitors (ChEI). The major therapeutic effect of ChEI on AD patients is to maintain cognitive function at a stable level during a 6-month to 1-year period of treatment, as compared to placebo. Additional drug effects are to slow down cognitive deterioration and improve behavioral and daily living activity. Recent studies show that in many patients the cognitive stabilization effect can be prolonged up to 24 months. This long-lasting effect suggests a mechanism of action other than symptomatic, and directly cholinergic. In vitro and in vivo studies have consistently demonstrated a link between cholinergic activation and amyloid precursor protein (APP) metabolism. Lesions of cholinergic nuclei cause a rapid increase in cortical APP and cholinergic synaptic function; the effect of such lesions can be reversed by ChEI treatment. A reduction in cholinergic neurotransmission, experimental or pathological, leads to amyloidogenic metabolism and contributes to the development of neuropathology and cognitive dysfunction. To explain the long-term effect of ChEI, for which evidence is available on an experimental as well as clinical level, a mechanism based on beta-amyloid metabolism is postulated. The question whether cholinergic stabilization implies simply slowing down progression of disability or also involves delay of disease progression is discussed.
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CHOLINERGIC THERAPY OF ALZHEIMER'S DISEASE. HOW DID IT START?

There are presently three possible modalities of pharmacological treatment of Alzheimer's disease (AD) (Fig. 1); the most explored has been the cholinergic approach.

Basic as well as clinical knowledge of the cholinergic system, its normal function, and dysfunction form the theoretical base of the therapy of AD with cholinesterase inhibitors (ChEI) (1). This strategy was developed considering that: a) cholinergic neurons and synapses undergo early and selective damage, as demonstrated by a decrease in acetylcholine (ACh) synthesis and nicotinic receptor binding (1); b) a steady-state rise in synaptic ACh levels following cholinesterase (ChE) inhibition produces a symptomatic short-term cognitive benefit which can be demonstrated experimentally in animals and humans; c) because this effect is short-lasting and symptomatic in nature, efficacy of ChEI should not persist beyond cessation of the central nervous system (CNS)-ACh elevation resulting from ChE inhibition; d) based on these three conditions, one can predict that early, mildly affected patients should respond best to ChEI therapy, while severely impaired patients with extensive cholinergic dysfunction would not benefit from this treatment; e) finally, clinical benefits should be limited to cognitive (memory), non-behavioral symptomatic improvement (Table 1).

Recent clinical data seem to contradict such assumptions (Table 2). First, neocortical cholinergic deficits such as decrease in choline-acetyltransferase (ChAT) and acetylcholinesterase (AChE) enzymatic activity, which are characteristic of severely demented patients, are not clearly apparent in individuals with

Key words: Alzheimer's disease, amyloid precursor protein (APP), beta-amyloid, cholinergic stabilization, cholinesterase inhibitors (ChEI).

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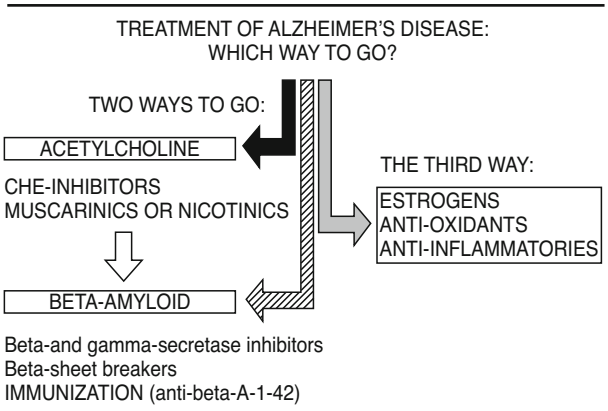

Figure 1 - *Pharmacological treatment of Alzheimer's disease present approaches.*

early mild AD (2). Significant cholinergic enzymatic deficits are not demonstrable until relatively late in the course of the disease (2). On the other hand, decreases in ChAT activity in the frontal and parietal cortex and in the hippocampus correlate to losses of cognitive domain scores (MDRS, Mattis Dementia Rating Scale and MMSE, Mini Mental State Examination) (3). The clinical effect of ChEI seen at early and mild stages of the disease suggests that the cholinergic system may be hypo-functional, or that parameters other than enzyme activity levels are impaired. Demonstrated deficits are: reduced ACh biosynthesis; decreased storage and impaired release of ACh, and cholinergic receptor defective either in number (nicotinic) or in function (muscarinic) (1).

To support adequate synthesis and hydrolysis of ACh under non-physiological conditions, cholinergic enzymes are in excess concentrations in the brain (1). This implies that patients at severe stages of the disease showing extensive cholinergic damage would constitute a target for cholinergic treatment. Trials designed specifically to investigate indications for asymptomatic early-phase or severe late-phase patients are in progress. The preliminary data available suggest that pharmacological interventions can improve cognitive function in the range from very mild and minimally cognitively impaired AD patients (cases showing memory impairment only) to severe cases (MMSE<10). Treatment duration can be extended to two-three years, which correspond approximately to one third of the natural history of the disease, thus raising the question: is such a prolonged effect of ChEI only symptomatic, or are ChEI disease-modifying drugs? Recent data, which are summarized in this paper, support the second alternative. The clinical effect seems to be stronger in more advanced cases; moreover, treatment benefit seems to be more pronounced and more long-lasting at higher doses. The clinical effect of ChEI is measurable three-four weeks following interruption of the treatment (washout period) despite the fact that ChE inhibition is strongly diminished or no longer present (4). In this case, new clinical effect can be demonstrated as an improvement in ADAS-Cog scores when treatment is resumed after being suspended for several weeks (3-6 weeks). It seems difficult to explain this stabilizing effect in terms of symptomatic mechanisms (Table 2). Evidence accumulated from experiments in animals and neuronal cell lines suggests a different explanation (5-7).

Table 1 - *Basic assumptions for a cholinergic strategy. Are they correct?*

1. Brain cholinergic neurons and synapses are damaged very early in the disease process which results in a decreased level of acetylcholine.
2. Early, mildly-affected patients will benefit maximally from ChEI treatment.
3. Treatment benefit will be short-term, symptomatic and mainly cognitive.
4. Drug effect will not persist beyond cessation of ChE inhibition.

ChEI: Cholinesterase Inhibitors; ChE: Cholinesterase.

CLINICAL DATA SUPPORT A STABILIZING EFFECT OF CHOLINESTERASE INHIBITORS

The benefit of ChEI treatment was previously considered to be exclusively symptomatic and cognitive. It has now been demonstrated that improvement involves cognitive as well as behavioral symptoms (8, 9). The cognitive improvement is significant up to 12 months (Fig. 2), and several clinical studies have

Table 2 - *Evidence that ChEI clinical effect may not only be symptomatic.*

1. Cognitive deterioration progresses more slowly in treated than in untreated patients. This effect is dose-dependent.
2. The clinical effect may last for several weeks after drug discontinuation while cholinesterase inhibition in CSF is low or even absent.
3. The clinical effect is seen also in early, mildly affected patients with little cholinergic impairment.
4. The strongest clinical effect is seen in rapidly progressing patients.
5. Relatively more advanced patients respond better than early mild cases.

ChEI: Cholinesterase Inhibitors; CSF: Cholinergic Synaptic Function.

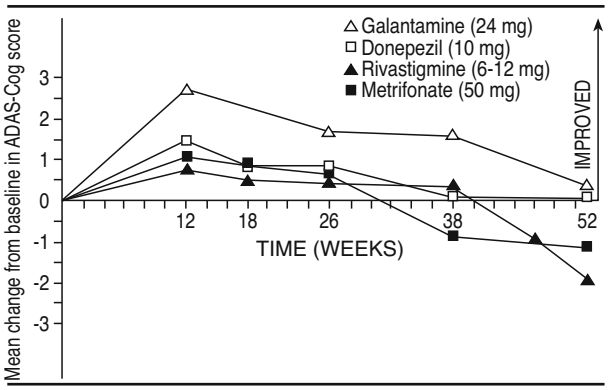

Figure 2 - *Stabilization effect of 12-month treatment with four cholinesterase inhibitors. The patients change little cognitively from baseline during this period (10, 13, 15, 18).*

demonstrated that the drug effect can be seen for as long as 2 years in many patients (Table 3). This long-term effect translates into an improved activity of daily living for the patient, and a reduced emotional impact for the caregiver, as well as a reduction in care costs.

The six-month data available for six ChEI suggest that patients treated with the active compound change little cognitively from baseline at the beginning of the trial to the end of the trial (19, 20). Studies that approximate a randomized-start design suggest that treatment with ChEI may delay cognitive deterioration (21). Two-year open-label data from a donepezil trial reveal a decline in ADAS-Cog from baseline that is 50% lower than the predicted value; untreated patients progress more rapidly than treated ones, and the treatment effect seems to be related to the dose (21). Average annual rate of decline for patients with a higher dose of rivastigmine is almost 50% lower (4.5 ADAS-Cog points/year) than that of patients treated with a lower dose (8.2 ADAS-Cog points/year) (21).

Increasing the dose of rivastigmine reduces the rate of cognitive decline over a 3-year period, suggesting a reduction in the rate of progression of cognitive deterioration (10, 21). Clinical data also indicate that rapidly progressing patients show the strongest drug effect, therefore, both disease stage and dose of the ChEI seem to play a role in altering the course of the disease (21).

Stabilization of cognitive deterioration suggests either a protective and structural effect, or a long-term improvement in the cholinergic synaptic function. The gradual return to the predicted deterioration-line after drug wash-out also suggests additional non-cholinergic effects. The long-term clinical effect could be related either to cholinesterase inhibition through the active site of the enzyme (5-7), or to non-cholinergic properties through interaction with a site close to the peripheral anionic binding site of the enzyme (22).

LONG-TERM STABILIZING EFFECTS OF CHOLINESTERASE INHIBITORS

Recent data from 12-24 month open trials and one randomized placebo-control trial suggest that optimization and maintainance of clinical effects for one year or more is a feasible goal in many patients (Table 3). Figure 2 reports the effect on the mean change in ADAS-Cog score of a 12-month treatment with three ChEI presently in clinical use: donepezil (17), galantamine (18) and rivastigmine (10). The data at twelve months show either a small or no difference from the baseline. The results of several clinical studies (placebo-controlled and open label) for periods longer than one year (up to 3 years) are re-

Table 3 - *Long-term efficacy of five cholinesterase inhibitors in AD patients.*

Reference No.	Drug	No. of patients	Max. treatment duration/years	Measures	Benefit difference
11,17	Donepezil	1600	2	ADAS-Cog	positive****
46		133	4,9	ADAS-Cog, CDR	positive
15		431**	1	ADFAC-CDR	positive
16		286***	1	GBS-MMSE	positive
12	Tacrine	25	1	MMSE-EEG	positive
13	Metrifonate	432*	1	ADAS-Cog, CIBIC+, MMSE	positive
10	Rivastigmine	2149	2	ADAS-Cog, MMSE, CIBIC+, GDS,	positive
14	Galantamine	44	3	ADAS-Cog	positive
18		636	1	ADAS-Cog	positive

Bernhardt and Woelk, 2000 (13)*, Winblad et al., 1999 (15)**, and Mohs et al., 1999 (16)*** are prospective, placebo-controlled, double-blind studies. Total number of patients: 4258. ****Positive indicates statistically significant clinical improvement from baseline.

ported in Table 3 (10-18). These data indicate that benefit differences can be maintained in a number of patients for up to 12-24 months by five different inhibitors (donepezil, tacrine, metrifonate, rivastigmine and galantamine). In terms of global improvement in the ADAS-Cog score, this may sum up to a total 15-20 point gain, which represents an 18-24 month difference in disease history from placebo-treated patients. How to interpret this improvement? Is it the result of slowing down the increase of disability, or is it an expression of delaying progression of the disease? This question is similar to that asked in reference to the effect of MAO-B inhibitors such as selegyline with regard to Parkinson disease.

BASIC RESEARCH DATA SUPPORTING NON-SYMPTOMATIC EFFECT OF CHOLINESTERASE INHIBITORS

The amyloid precursor protein (APP) pathway that generates beta-amyloid (beta-A) is regulated by the sequential action of three enzymes (alpha, beta and gamma secretases). Alpha secretase cleaves APP within the beta-A sequence, and releases soluble N-terminal non-aggregating fragments (sAPP) (Fig. 3). Numerous studies have shown that the stimulation of sAPP release is associated with reduced formation of amyloidogenic peptides. Muscarinic-agonist-induced sAPP secretion through activation by carbachol of m_1 and m_3 (but not m_2 and m_4) receptor subtypes increases sAPP release in human embryonic cell lines (23). Activation of the pathway that cleaves APP decreases the release of beta-A fragments, and may slow down amyloid formation in the brain. On the basis of the results obtained in superfused rat cortex slices demonstrating an increased release of sAPP in response to muscarinic stimulation, we proposed an effect of ChEI on sAPP secretion acting through the same pathway (5) (Fig. 3).

Racchi et al. (6) using neuroblastoma cells, and Pakaski et al. (7) using primary cultures of rat basal forebrain neurons have shown that short-term treatment with reversible and irreversible ChEI such as ambenomium, and metrifonate or its metabolite DDVP, increases sAPP release into the conditioned media, and elevates protein kinase C (PKC) levels. These studies demonstrated that this effect on APP is consistent with AChE inhibition, and with indirect muscarinic-mediated cholinergic stimulation. In addition, short-term or long-term stimulation does not result in changes in APP mRNA expression either in cortical slices or neuroblastoma cells (6, 20), nor in a down-regulation of the response to cholinergic stimulation of muscarinic receptors (6). These results suggest that

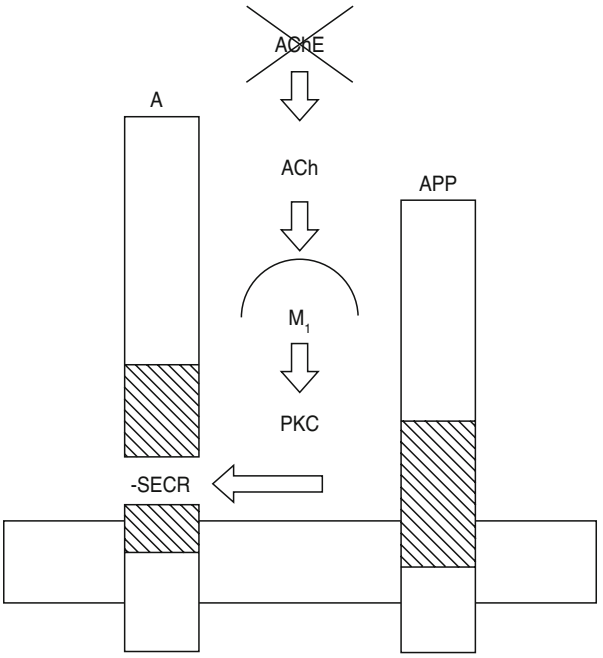

Figure 3 - *Secretion of sAPP can be increased in the brain by direct stimulation of m_1 muscarinic receptors, or indirectly through cholinesterase inhibition producing an increase in acetylcholine and subsequent protein phosphorylation (PKC). This effect might concomitantly decrease the production of potentially amyloidogenic Beta-A peptides and slow down progression of the disease.*
ACh: Acetylcholine; PKC: Protein Kinase C; APP: Amyloid Precursor Protein; -Secr: Alfa-Secretases; -A: beta-Amlyoid; M_1: m_1 muscarinic receptors.

ChEI promote the non-amyloidogenic route of APP processing through a stimulation of alpha-secretase activity mediated by PKC (Fig. 3).

This demonstrated feature of ChEI and of muscarinic agonists, and their ability to enhance the release of non-amyloidogenic soluble derivatives of APP *in vitro* and *in vivo* suggests a slowing down in the formation of amyloidogenic compounds in the brain (1).

EFFECT OF CHOLINERGIC STIMULATION ON BETA-A BRAIN METABOLISM?

Selective muscarinic (m_1) direct activation of alpha-secretase activity accelerates APP processing, and consequently decreases the generation of beta-A peptides in cellular model systems (6, 7, 23). A reduction in both total beta-A and beta-1-42 peptide levels in the cholinergic synaptic function (CSF) is seen in AD patients treated for 4 weeks with either AF102B,

Table 4 - *Effect of ChEI on CSF and RBC AChE activity (1).*

Ref. No.	Drug	Route of administration (dose)	AChE activity (percent)		Time
			CSF	RB	
1	physostigmine	icv (1 μg)	30	100	5 minutes
1	physostigmine	icv (8 μg)	15	100	5 minutes
10	rivastigmine	oral (3 mg)	70	90	2 hours
30	rivastigmine	oral (12 mg)	60	21**	12 months
31	tacrine	oral (80-160 mg)	150	75	12 months
32	metrifonate	oral (2.9 mg/kg w.)	40-100	33	6 months
33	metrifonate	oral (2.9 mg/kg w.)	92	35	24 months*

* Open label (33), ** ChE activity in plasma (30).
CSF: Cholinergic Synaptic Function; RBC: Red Blood Cells; AChE: Acetylcholinesterase; ChE: Cholinesterase; icv: intracerebral ventricular.

or talsaclidine in placebo-controlled trials (24). Both drugs are selective m_1 agonists. The decrease in total CSF beta-A is in the range of 10-40% in 80% of patients (24). No changes are seen in either total tau or phosphorylated tau CSF levels. Levels of total beta-A do not change following treatment with the ChEI physostigmine, or with the anti-inflammatory drug hydroxychloroquine. It is interesting to note that physostigmine, in contrast to metrifonate, does not show any effect on sAPP secretion in cell lines (25). In rat cortical slices, the physostigmine effect is dose-dependent (5). Tacrine, on the other hand, was shown to increase sAPP release and decrease levels of beta-A, beta-1-40 and beta-1-42 in both cell lines and cortical slices (25, 26).

Lahiri et al. (25) demonstrated that levels of soluble beta-1-42 were reduced by 20-25% when human neuroblastoma cells were treated with either 3,4 diaminopyridine, metrifonate or tacrine, but were unchanged with physostigmine. In these experiments, levels of shorter beta-A species were found to be 10-fold higher than longer and potentially amyloidogenic beta-1-42 species (25). Metrifonate treatment resulted in the lowest percent accumulation of the beta-1-42 species relative to the total secreted into the conditioned media than any other drug tested. It was also found that the effects of ChEI on sAPP release do not depend on their selectivity for either AChE or BuChE.

On the basis of the results obtained *in vitro* and *in vivo*, ChEI can be classified into two groups: the first group with little or no effect on APP processing as exemplified by physostigmine, and the second group represented by drugs such as tacrine and metrifonate that increase APP release and decrease beta-A levels. The fact that anti-amyloid properties are not common to all ChEI poses the question of optimization of cholinergic properties combined with anti-amyloid effects as a challenge for future drug development (1).

ChE BRAIN INHIBITION AND CLINICAL EFFECT: A CRITICAL RELATION

The cognitive effect (ADAS-Cog) seen with most ChEI becomes statistically significant after 2-3 weeks of treatment (Fig. 2). This delay may be due to attenuation of the placebo effect. In most patients, some decrease in the clinical effect is observed following a period of 30-36 weeks of treatment. Does this decrease depend on progressive patient deterioration, or on other factors such as lower clinical efficacy of ChEI medication? How is the long-term clinical effect maintained if enzyme inhibition is progressively decreasing?

The data suggest that tolerance to repeated doses of ChEI, combined with patient deterioration, may contribute to attenuate the clinical effect of the drug. There is a vast literature addressing the phenomenon of tolerance to both single and repeated doses of ChEI (27). Adaptation due to decreased effect of ChEI is supported by behavioral as well as toxicological studies (27). Tolerance to repeated doses of ChEI might be explained by two mechanisms (27). First, a reduction in sensitivity due to ACh elevation causes a decrease in the number (down-regulation) of muscarinic and/or nicotinic receptors. Alzheimer patients at advanced disease stages show a deficit in synthesis and levels of Ach, and a decrease in nicotinic (but not muscarinic) receptor number (28).

A second, more likely explanation, is the induction of new enzyme synthesis by increased AChE gene expression at nerve terminals (29). Direct stimulation of m_1 muscarinic receptors or ACh itself may be the signal for increased gene expression. Von der Kammer et al. (29) demonstrated activation of m_1 increased transcription from Egr-dependent promoters, including the AChE promoter. This effect is reflected by an increased level of AChE activity (not an inhibition!) in the CSF of long-term treated patients (1, 10, 30-33) (Table 4).

Table 5 - *Acetylcholinesterase-beta-amyloid relationship in AD brain.*

ACETYLCHOLINESTERASE:
1. Is associated with the amyloid core of neuritic plaques (37). The hydrophobic site of aggregation is distinct from the enzymatic active site (38).
2. Is abnormally glycosylated (39, 40) and its expression is stimulated by beta-amyloid (23, 40).
3. Is increased within and around the amyloid plaques (37) and promotes aggregation of amyloid beta-peptide fragments (41).
4. Is increased in the brain of transgenic mice expressing the c-terminal fragment of the beta-amyloid precursor protein (42).
5. Nucleus basalis lesions increase synthesis of beta-amyloid (43).

Stimulation of AChE release from CNS neurons into the CSF by the action of ChEI has been invoked by Bareggi and Giacobini (34). Increased AChE activity in the CSF was demonstrated by Mattio et al. (35) in dogs chronically administered high doses of physostigmine intraventricularly. An example of upregulation of AChE activity is seen in the CSF of tacrine-treated patients (80-160 mg for 12 months), as reported by Nordberg et al. (31) (Table 4). In this study, no change was observed in AChE inhibition in RBC, while a 50% increase (sic) in AChE (but not BuChE) activity was seen in the CSF. Other observations indicate that an upregulated synthesis of both AChE and BuChE may occur in dogs and rats treated with various types of ChEI (1). Metrifonate, an organophosphate ChEI, does not produce AChE upregulation in the brain or RBC of rats treated for 12 weeks (36). In AD patients, RBC AChE inhibition is the same at 2, 8 and 12 weeks following oral administration (0.65-2.0 mg/kg/day) of metrifonate (33). This is in agreement with the data on CSF following rivastigmine treatment, but in contrast with the effect of tacrine on CSF ChE (30) (Table 4).

In conclusion, there seems to be a difference in the production of tolerance and enzymatic induction among different compounds. It is surprising that in certain cases clinical efficacy can be maintained despite low enzymatic effect, implying mechanisms other than ChE inhibition.

HOW TO EXPLAIN A POSSIBLE DISEASE-MODIFYING EFFECT OF CHOLINESTERASE INHIBITORS?

Table 5 summarizes the special relationship between AChE and beta-amyloid in the brain of AD patients (1). Accordingly, AChE, which is present in a glycosylated form associated with the amyloid core of neuritic plaques (37), is stimulated in its synthesis by beta-amyloid. AChE stimulates beta-amyloid accumulation in or near to the plaque (22, 40) (Fig. 4).

One first explanation for a long-term neuroprotective effect of ChEI is an interaction of the beta-A cycle with ChEI, promoting the release of soluble forms of beta-APP, and the reduction of amyloidogenic forms. Such a mechanism implies the regulation of alpha secretase activity as the common final effector of PKC-dependent modulation of APP metabolism acting through a muscarinic m_1 receptor, as supported by experimental evidence (5-7, 20, 23). Secretion of APP soluble forms related to ChE inhibition has been demonstrated *in vitro* and *in vivo* in the CSF of NBM lesioned rats (rivastigmine and phenserine) (44), neuroblastoma cells for metrifonate (6), rat cortex slices (metrifonate) (5), rat basal forebrain cell cultures (metrifonate and ambenomium) (7), and in human neuroblastoma cells for several ChEI (25, 45).

A second hypothesis is based on the data showing that the AChE molecule may interact with beta-amyloid through a hydrophobic site close to the peripheral anionic binding site (PAS), promoting amyloid fibril formation (22). In addition, AChE is incorporated into senile plaques *in vitro* by forming macromolecular complexes with the growing beta-amyloid fibrils (44). AChEI binding to PAS may reduce this formation. Both hypotheses need clinical confirmation utilizing CSF markers to monitor brain beta-amyloid levels and metabolism in patients treated with ChEI for a long term. Studies are in progress to verify such an effect in patients.

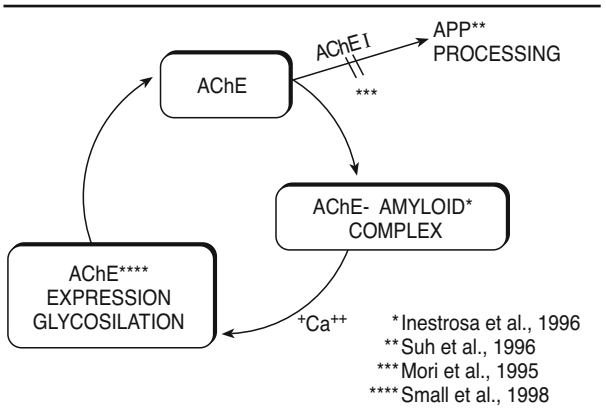

Figure 4 - *Proposed beta-amyloid cycle: AChE co-localizes with beta-amyloid and accelerates beta-amyloid formation and deposition in AD brain. Beta-amyloid increases AChE in the brain. Inhibition of AChE activity inhibiting APP release reduces beta-amyloid deposition. This mechanism could contribute to the patient long-term cognitive stabilization seen during AChEI treatment.*
AChE: Acetylcholinesterase; APP: Amyloid Precursor Protein.

The longest clinical study on the effect of a ChEI was performed as an extension trial lasting almost 5 years. The results demonstrate treatment benefits on cognition and global function in patients progressing from mild to moderate AD, and from moderate to severe AD over a period of 4, 9 years (46). The mean annual rate of decline in ADAS-Cog and CDR (Clinical Dementia Rating) of patients receiving the ChEI was significantly less over the first through the third year of treatment than might be predicted had this cohort of patients not been treated (46). This long-term efficacy supports the concept of a disease-delaying effect of this class of drugs.

CONCLUSIONS

In vitro as well as *in vivo* studies have consistently demonstrated a link between cholinergic activation and APP metabolism. A lesion of the cholinergic nuclei (nucleus basalis Meynert) causes a rapid increase in APP in the neocortex and CSF of rats (43). The effect of such lesions can be reversed by treatment with ChEI (44). A reduction in cholinergic neurotransmission, experimental or pathological, leads to amyloidogenic metabolism in the brain, and contributes to the neuropathology and cognitive dysfunction. Cholinesterase inhibitors may interact with both mechanisms, stabilizing the patient. This interpretation establishes a relationship between the effects of cholinergic drugs with a muscarinic mechanism of action, such as ChEI, and muscarinic agonists with treatments specifically targeted to decrease the beta-amyloid burden in the brain (Fig. 1).

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