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Structure–activity relationships of acetylcholinesterase inhibitors: Donepezil hydrochloride for the treatment of Alzheimer's Disease*

Hachiro Sugimoto†

Tsukuba Research Laboratories, Eisai Co., Ltd, 5-1-3 Tokodai, Tsukuba-shi, Ibaraki-ken, Japan 300-2635

Abstract: A wide range of evidence show that acetylcholinesterase (AChE) inhibitors can interfere with the progression of Alzheimer's Disease (AD). The successful development of these compounds was based on a well-accepted theory that the decline in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission. The earliest known AChE inhibitors, namely, physostigmine and tacrine, showed modest improvement in the cognitive function of Alzheimer's patients. However, clinical studies show that physostigmine has poor oral activity, brain penetration and pharmacokinetic parameters while tacrine has hepatotoxic liability. Studies were then focused on finding a new type of acetylcholinesterase inhibitor that would overcome the disadvantages of these two compounds. Donepezil hydrochloride inaugurates a new class of AChE inhibitors with longer and more selective action with manageable adverse effects. Currently, there are about 19 new Alzheimer's drugs in various phases of clinical development.

ALZHEIMER'S DISEASE

Alzheimer's Disease, discovered by Dr Alois Alzheimer in 1907, is described as a degenerative disease of the central nervous system (CNS) characterized especially by premature senile mental deterioration. AD patients exhibit marked decline in cognitive ability and severe behavioral abnormalities such as irritability, anxiety, depression, disorientation, and restlessness. AD is a progressive disease, i.e. the onset of the disease may show mild symptoms but these symptoms will sooner or later become more and more severe until the patient loses his or her capacity to handle normal daily activities. While AD is commonly regarded as a senile disease, the symptoms can also manifest in presenile individuals.

CHOLINERGIC HYPOTHESIS

The most consistent neurotransmitter-related change in the brain of an AD patient is the dramatic decrease in cholinergic innervation in the cortex and hippocampus due to the loss of neurons in the basal forebrain. This fact has been confirmed in a large number of animal and human studies. This loss of cholinergic neurons and the associated decrease in levels of cholinergic neurotransmission have been associated with the cognitive impairment seen in AD patients [1].

The above findings led to the development of the cholinergic hypothesis. Simply stated, the cholinergic hypothesis proposes that the cognitive loss associated with AD is related to decreased cortical cholinergic neurotransmission. Therefore, it is presumed that increasing cholinergic transmission may enhance cognitive function [1,2].

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† *Correspondence*: h1-sugimoto@eisai.co.uk

The cholinergic theory has provided the rational basis for therapeutic developments in AD. Based on this theory, six classes of drugs have been developed to enhance cholinergic deficit in AD patient. These are:

(a) Cholinesterase inhibitors (ChEI), which block the AChE enzyme thereby invigorating cholinergic activity to enhance cognitive function.

(b) Choline precursors, such as phosphatidylcholine, aimed at increasing the bioavailability of choline.

(c) Acetylcholine (ACh) releasers, which should facilitate the release of ACh from presynaptic end terminals.

(d) M1 and M3 receptor agonists, which mimic ACh on postsynaptic end terminal receptors.

(e) M2 and M3 receptor antagonists, generally presynaptic (autoreceptors), which regulate ACh release via negative feedback.

(f) Nicotinic agonists or substances having nicotinic-like effects, which should enhance ACh release.

Among the above pharmacological agents, AChE inhibitors seem to be the most effective method to improve cholinergic deficit thus reducing the symptoms of the disease [3].

DONEPEZIL HYDROCHLORIDE (E2020)

Donepezil hydrochloride (E2020) (Fig. 1) is the second drug approved by the US FDA for the treatment of mild to moderate AD. It is a new class of AChE inhibitor having an *N*-benzylpiperidine and an indanone moiety which shows longer and more selective action. It is now marketed in the US and in some European and Asian countries under the trade name of Aricept®. In Japan, Aricept® is now under application to the Ministry of Welfare.

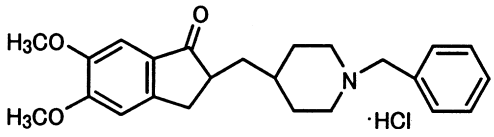

Fig. 1 Donepezil hydrochloride.

The research on E2020 started in 1983. First of all, our group in Eisai Co., Ltd, started to develop tacrine derivatives. However, we failed to develop a non-toxic tacrine derivative. Through random screening, we encountered *N*-benzylpiperazine derivative (compound **1**) which was then originally being synthesized in the study of anti-arterial sclerosis. Our tests showed that the acetylcholinesterase activity of *N*-benzylpiperazine derivative was 12 600 nM at IC_{50} in rat brain homogenate. This was not very strong but the compound's novel structure was very promising. We decided to use *N*-benzylpiperazine derivative as the seed compound and synthesized about 700 derivatives.

Our succeeding experiments showed a dramatic increase in anti-acetylcholinesterase activity when *N*-benzylpiperazine was replaced with *N*-benzylpiperidine (**2**) (Fig. 2). It was a quantum leap in our investigation. Our next challenge was to replace the ether moiety with an amide moeity (**3**). This process also increased anti-acetylcholinesterase activity.

On the basis of these findings, we synthesized benzamide derivatives. We later on discovered that benzylsulfonyl derivative (**4**) was the most potent AChE inhibitor with an anti-AChE activity 21 000-fold greater compared to the seed compound [4,5]. However, our excitement was short-lived because we found that this compound has a very poor bioavailability rate and has a short duration of action and therefore could not be a candidate for clinical testing. But this benzylsulfonyl derivative has a novel chemical structure and has a selective affinity to AChE, making it a very attractive lead compound.

Our next strategy in drug design was the replacement of the amide moiety with ketone moiety (**7**). This approach maintained the AChE activity of the compound. Furthermore, this cyclic-amide derivative (**6**) showed enhanced inhibitory action. On the basis of these results, an indanone derivative (**8**) was designed. The resulting AChE activity was moderate, but we achieved longer duration of action. Subsequently, various indanone derivatives were synthesized and tested for anti-AChE activity. Among the indanone derivatives that were developed, donepezil was found to be the most valence compound (Fig. 3) [6].

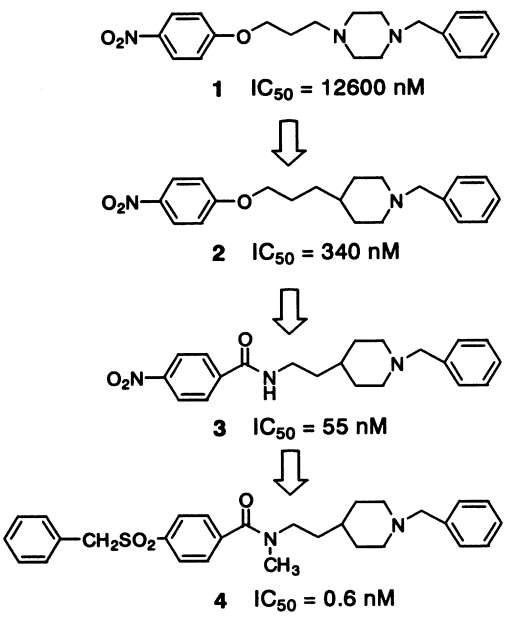

Fig. 2 Leading compounds.

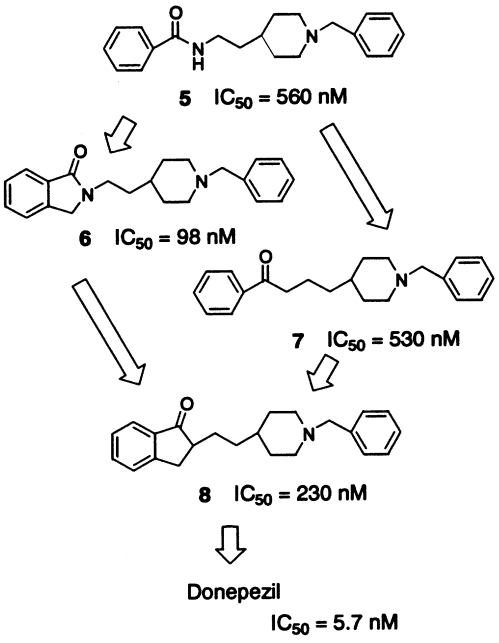

Fig. 3 New leading compounds.

PRECLINICAL PHARMACOLOGY OF DONEPEZIL HYDROCHLORIDE (E2020)

The following experiments were designed to evaluate the properties of donepezil, a new cholinesterase inhibitor, with respect to its effect on the central cholinergic system. The conventional ChE inhibitors such as tacrine and physostigmine (PHY) were used as reference compound in some experiments.

Effects on cholinesterase activity

(a) The comparative specificity of donepezil, tacrine, and PHY for brain AChE Activity

The initial experiments were designed to determine the relative *in vitro* inhibitory effects of donepezil

on the activities of AChE and butyrylcholinesterase (BuChE, pseudocholinesterase) in comparison with two recognized cholinesterase inhibitors, PHY and tacrine. Rat brain homogenates were used as the source of AChE and rat palsma served as the source of BuChE. ACh was used as the substrate for AChE and butyrylcholine (BuCh) was the substrate for BuChE. Both enzyme preparations were incubated with several concentrations of each inhibitor. The results, expressed as IC_{50} values, are shown in Table 1.

Table 1 Inhibitory effects of E2020 and reference compounds on rat brain AChE and rat plasma BuChE *in vitro*

Compound	IC_{50} (nM)		Ratio of IC_{50}s (BuChE/AChE)
	AChE Activity	BuChE Activity	
E2020	5.7 ± 0.2	7138 ± 133	1252.0
PHY	0.68 ± 0.02	8.1 ± 0.3	11.9
Tacrine	80.6 ± 2.5	73.0 ± 0.9	0.9

Values represent the mean ± SE from four dose–response curves for each test drug.

(b) The effect of oral administration of donepezil and other cholinesterase inhibitors on the cholinesterase activity of treated animals

In order to determine the comparative effect of cholinesterase inhibitors in vivo, the compounds were administered orally at doses from 1 to 60 mg/kg to male Wistar rats. One hour after administration, the rats were sacrificed and the brain (excluding the cerebellum) was frozen. AChE activity was later measured in a brain homogenate using acetylthiocholine as a substrate. The results of this in vivo treatment (Fig. 4) indicate that all three compounds caused a dose-dependent inhibition of brain AChE activity, and that donepezil appeared to be a more potent inhibitor than either PHY or tacrine [7].

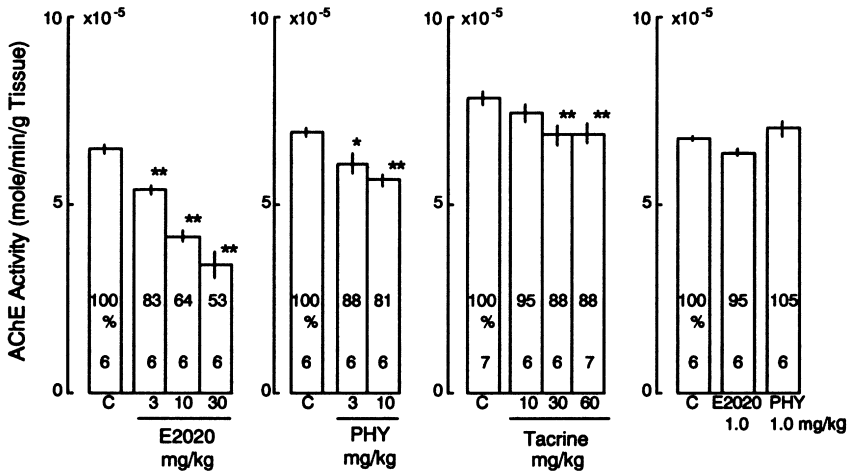

Fig. 4 Effects of oral administration of E2020, PHY and tacrine on rat brain AChE activity *ex vivo*. Each column denotes ± SE. *, $P < 0.05$, **$P < 0.01$. The top numbers in each column represent the percentage inhibition relative to control.

Effects of donepezil on brain acetylcholine concentrations

(a) The effect of donepezil and tacrine on ACh concentration in the cerebral cortex of animals with cerebral cholinergic hypofunction

Since donepezil was designed for use under circumstances in which the concentration of ACh is below normal level, it was tested, along with tacrine, in a series of *in vivo* model systems in which the cortical cholinergic system is impaired.

In the study, the neurotoxin ibotenic acid was injected into the *nucleus basalis magnocellularis* region

of the rat brain. Destruction of this region, which innervates the cerebral cortex, causes a decrease in the concentration of acetylcholine in the cerebral cortex. Two to three weeks after injection, the animals were given an oral dose of either donepezil or tacrine. One hour later, the animals were sacrificed by whole-head microwave irradiation, and the concentration of ACh in the cerebral cortex was determined by HPLC analysis. The results (Fig. 5) indicate that exposure to ibotenic acid significantly decreased the concentration of ACh in the cerebral cortex, and that treatment with either donepezil (1.25 to 10 mg/kg) or tacrine (5 to 20 mg/kg), caused a dose-dependent increase in cortical ACh. In this model system, donepezil appears to be a more potent agent than tacrine.

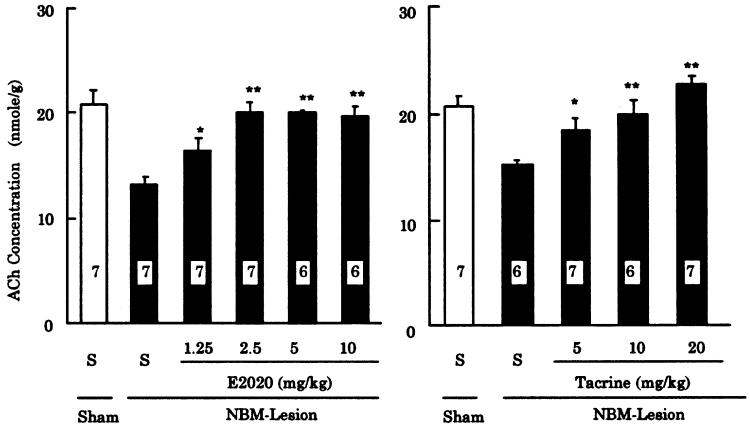

Fig. 5 Effects of E2020 and tacrine on ACh concentration in the cerebral cortex of rats with ibotenic acid-induced NBM lesion. $*P<0.05$, $**P<0.01$ vs. NBM-lesioned rats (Dunnett's *t*-test). S: Saline; Sham: Sham operation.

Effect of donepezil in behavioral models of cholinergic hypofunction

On the basis of the previous studies, it is apparent that donepezil is a relatively specific inhibitor of AChE which can increase the concentration of ACh in ACh-deficient animals.

(a) The effect of treatment with donepezil on the behavior of functionally impaired animals

The study was designed to evaluate the effects of donepezil and tacrine on a passive avoidance task in animals with lesions in the *nucleus basalis magnocellularis* (NBM) [8]. The NBM was destroyed in test animals by bilateral injection of ibotenic acid. After one week, NBM-lesioned and sham-operated animals were placed in a passive avoidance box consisting of light and dark compartments, and trained, using electric shock, to avoid entry into the dark compartment. One hour prior to training, they were given either donepezil, tacrine or saline orally, and tested 24 hours later to determine whether they remembered their training. Retention (memory) was measured by the amount of time each animal waited before entering the dark compartment (response latency). Animals which retained the training, i.e. memory of the electric shock, had longer latency times. As shown in Fig. 6, sham-operated animals had a response latency of approximately 400 s, and lesioned animals treated with saline had a latency of approximately 100 s, indicating a decrease in their ability to retain the training. Lesioned animals treated with donepezil at doses from 0.125 to 1 mg/kg showed a statistically significant increase in latency. Animals treated with tacrine at doses from 0.25 to 1 mg/kg showed increases in response latency at 0.5 mg/kg, but this increase did not achieve statistical significance.

These results indicate that donepezil is capable of enhancing the retention of training (memory) in animals with cholinergic hypofunction.

CLINICAL STUDIES OF DONEPEZIL HYDROCHLORIDE

US multicenter study phase II

A double-blind, placebo-controlled, randomized trial 1, 3 and 5 mg donepezil in 141 patients was reported in 1996. A 12-week double-blind phase was followed by a-week single-blind placebo washout.

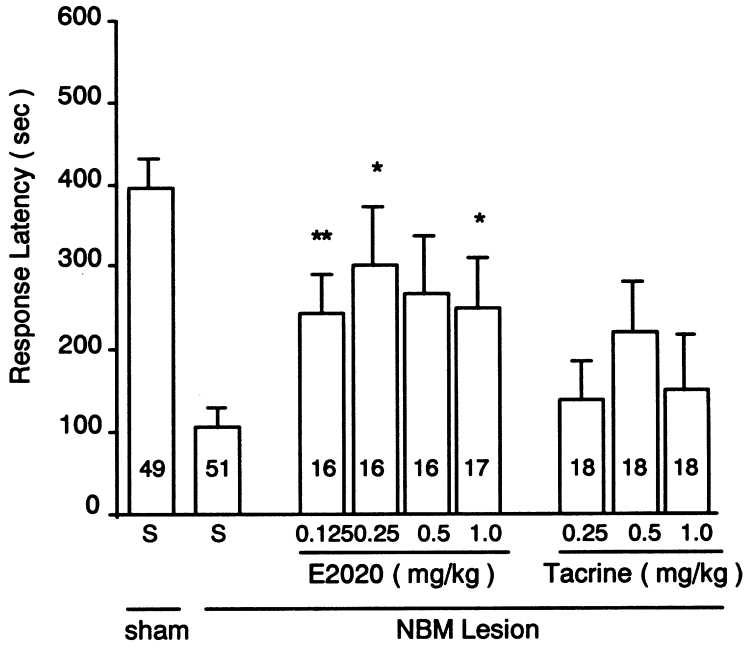

Fig. 6 Effects of E2020 and tacrine on the latency of passive avoidance response in NBM-lesioned rats. $^{*}P < 0.05$, $^{**}P < 0.01$ (Mann–Whitney's *U*-test). S: Saline, Sham: Sham-operated rats, NBM Lesion: NBM lesioned rats.

Improvements in Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) and Mini-mental State Exam (MMSE) scores were reported; no changes were found in this study of short duration on the clinical global impression of change. However, a statistically significant correlation between plasma concentrations of donepezil and AChE inhibition was demonstrated. Moreover, there appeared to be a possible correlation between plasma drug concentrations and cognitive scores. Treatment-related side effects were comparable with all three doses.

30-Week phase III study

In this study, which was similar in design to the 15-week study, approximately 150 patients each were entered into donepezil 5 mg, donepezil 10 mg or placebo. The patients were followed for 24 weeks, followed by a 6-weeks wash-out [9]. Once again, there were statistical improvements in ADAS-cog in patients treated with both drug doses at 12 and 18 weeks. Clinician's Interview-Based Impression of Change plus caregiver assessment (CIBIC-Plus) scores also improved in both groups compared to placebo.

Safety

A high proportion of patients completed both of the phase III studies [10]. Five per cent of patients dropped out due to adverse events in placebo and low-dose donepezil groups, increasing to 13% in the higher, 10 mg dose groups. This greater drop-out at the 10 mg dose was thought to be due to the rapid titration, since in open-label studies, the frequency of drop-out at 10 mg is much lower if the titration is taken over 4–6 weeks. Most of the treatment-emergent adverse events were mild in intensity and lasted less than 2 days. As with other cholinesterase inhibitors, the most common side effects were nausea, diarrhea, insomnia, muscle cramps, vomiting and fatigue.

CONCLUSIONS

Given the fact that the etiology and pathogenesis of AD is still unclear, the development of a curative compound is markedly limited. This limitation is even more compounded by the unavailability of a true animal model of AD. The screening of new AChE inhibitors is performed with a battery of

pharmacological testings. But the question remains on whether the animal models are valid [10]. Appropriately validated animal models are important in the efficient and rational development of AChE inhibitors and other treatment compounds for AD.

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