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Donepezil Hydrochloride: A Treatment Drug for Alzheimer's Disease

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ABSTRACT: The role of the cholinergic system with respect to cognitive deficits characteristic of Alzheimer's disease (AD) has led to a number of studies focusing on the development of acetylcholinesterase (AChE) inhibitors as a drug for treating this disease. The earliest known AChE inhibitors, namely, physostigmine and tacrine, performed poorly in clinical trials (e.g., poor oral activity, brain penetration, and 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 and with manageable adverse effects. © 2000 The Japan Chemical Journal Forum and John Wiley & Sons, Inc.Chem Rec 1:63–73, 2001

Key words: Alzheimer's disease; acetylcholinesterase (AChE) inhibitor; donepezil hydrochloride; dementia; E2020

Introduction

Dementia has been a major public health problem of the aging population in developed countries for decades.[1] The most common cause of dementia in Western countries is Alzheimer's Disease (AD). In Japan, cerebro-vascular disease (CVD) was reported as the most common cause of dementia, affecting about 42% of the Japanese population. Alzheimer's disease ranks second, affecting about 32% of the population. However, with the increased "graying" of the population in Japan, the proportion of elderly individuals afflicted with AD is likely to increase.

AD individuals exhibit retrogression in their mental health functions, rendering them incapacitated and unable to perform ordinary daily activities. Elderly persons are the ones most often afflicted with this disease. However, evidence shows that individuals as young as 40 years old can also be afflicted with AD. This situation poses a great burden, not only in terms of health care budgets, but also because of the emotional and physical stress brought upon the families and caregivers of AD patients. At this point, it is worth sharing my own personal experience in caring for an elderly person who was incapacitated by a disease similar to AD. My own mother was afflicted with multi-infarct dementia. I had never imagined that a person who was once so vibrant and lucid would be debilitated by such a disease, which robbed her of her memory and physical capacity. This very poignant experience provided the stimulus for my study of antidementia agents, specifically targeting memory loss and incapacitation.

Unlike CVD, however, the true nature and mechanism of Alzheimer's disease is still unknown, making the development of treatment drugs a complex endeavor. Competing theories abound. One of the strongest is that the primary villain is a protein fragment called Aβ (A-beta) that forms plaque in AD-affected brains. Aβ results when a ubiquitous protein known as amyloid precursor protein is snipped to pieces by enzymes called secretases. Aβ is present in everyone, but when its disposal cannot keep up with its production, trouble may occur.[2]

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However, it is not yet clearly understood how the deposition of this protein is involved in AD-associated cell loss, or even if it is actually the agent responsible for the neuronal cell death observed in AD patients.[3] The disease is characterized by a profound loss of cholinergic cells, which accounts for most of the severe cholinergic deficiency in the cortex and hippocampus in the brains of Alzheimer's patients. Currently, this loss of cholinergic function is the only evidentiary finding responsible for cognitive decline. Hence, therapeutic development has focused on this theory.

Cholinergic System and Alzheimer's Disease

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, caused by the loss of neurons in the basal forebrain. This change has been confirmed in a large number of studies on animals and humans. The loss of cholinergic neurons and the associated decrease in the level of cholinergic neurotransmission have both been associated with the cognitive impairment seen in AD patients.[4]

Cholinergic Hypothesis

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 neurotransmission. Therefore, it is presumed that cognitive function may be enhanced by increasing cholinergic transmission.[4,5]

Cholinergic Enhancement Therapy

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

1. Cholinesterase inhibitors (ChEI), which block the AChE enzyme, thereby invigorating cholinergic activity to enhance cognitive function.
2. Choline precursors, such as phosphatidylcholine, aimed at increasing the bioavailability of choline.
3. ACh (acetylcholine) releasers, which should facilitate the release of ACh from presynaptic end terminals.
4. M1 and M3 receptor agonists, which mimic ACh on postsynaptic end terminal receptors.
5. M2 and M3 receptor antagonists, generally presynaptic (autoreceptors), which regulate the release of ACh via negative feedback.
6. Nicotinic agonists or substances having nicotine-like effects, which should enhance ACh release.

Among the above pharmacological agents, AChE inhibitors seem to be the most effective in improving cholinergic deficits and reducing the symptoms of the disease.[6]

AChE Action Mechanism

ACh is the most abundant neurotransmitter in the body and is the primary neurotransmitter in the brain, which is responsible for cholinergic transmission. The enzyme AChE plays a key role in the hydrolysis of the neurotransmitter ACh. AChE tends to become deposited within the neurofibrillary tangles and amyloid plaques associated with Alzheimer's disease. In a study by Inestrosa and Alvarez,[7] several cellular proteins, including AChE, were found to colocalize with β-amyloid (Aβ) deposits, and to promote the assembly of Aβ peptide into amyloid fibrils. In this context, preventing the aggregation of Aβ into plaques is another way to combat AD.

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A related study by Alvarez et al.[8] reported that the incorporation of AChE into Alzheimer's amyloid aggregates results in the formation of stable complexes that change the biochemical and pharmacological properties of the enzyme, causing an increase in the neurotoxicity of the β-amyloid fibrils. This suggests that AChE could play a pathogenic role in AD by influencing the process leading to amyloid toxicity and the appearance of AD. Analysis of the catalytic activity of the AChE incorporated into these complexes shows anomalous behavior reminiscent of the AChE associated with senile plaques, which includes a resistance to low pH, high substrate concentrations, and lower sensitivity to antiacetylcholinesterase agents.

Donepezil Hydrochloride (E2020)

The Discovery of Donepezil Hydrochloride

Donepezil hydrochloride (E2020) 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 Aricept®. In Japan, Aricept® was introduced on November 24, 1999.

As with many other drug development endeavors, the discovery of E2020 was a long and arduous process. My colleagues at the Tsukuba Research Laboratories (Eisai Co., Ltd.) and I had to battle many obstacles and exert painstaking efforts in search of a viable seed compound. Our research started in 1983, with the synthesis of tacrine derivatives, followed by the synthesis of about 30 derivative compounds. Despite our efforts, however, we failed to find a nontoxic tacrine derivative. Through random screening, we encountered an *N*-benzylpiperazine derivative (compound **1**), which was being synthesized in a study to combat arterial sclerosis. First, we used electric eel (EE) AChE. Compound **1** exhibited antiAChE activity of IC_{50} = 0.62 μ*M*. Using rat brain (RB) homogenate as the source of AChE, the activity of compound **1** showed IC_{50} = 12.6 μ*M*. If we had first used AChE of rat brain homogenate, we would not have discovered this compound. For about 1 year, we studied the structure-activity relationship (SAR) of phenyl ether derivatives. We synthesized about 100 compounds and measured their activities according to the method of Ellman et al.[9]

This is a summary of the SAR of phenyl ether derivatives (Fig. 1):

1. Replacement of ether group (1) with a methylene group (2) or a sulfur group (3) decreased activity.
2. Introduction of a bulky group (4) at the para-position of the phenyl group increased activity. 3. Replacement of piperazine moiety with piperidine (5) increased activity by 70 times over the original compound **1**. Our successive experiments showed a dramatic increase in antiAChE activity if *N*-benzylpiperazine was replaced with *N*-benzylpiperidine.

If the ether group was replaced with amide group (6), antiAChE activity increased. We synthesized about 200 com-

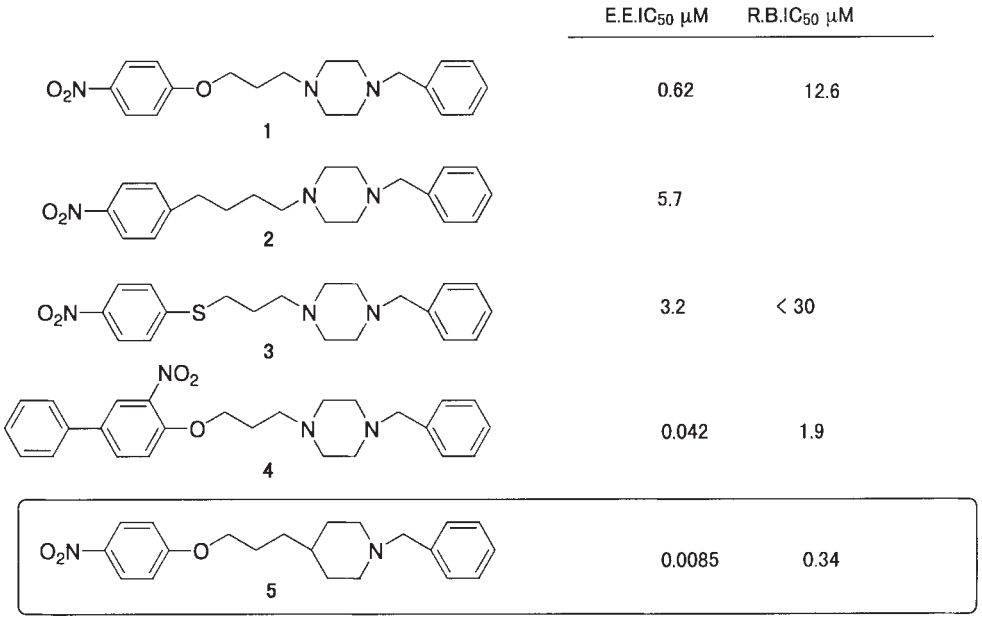

Fig. 1. SAR of phenyl ether derivatives.

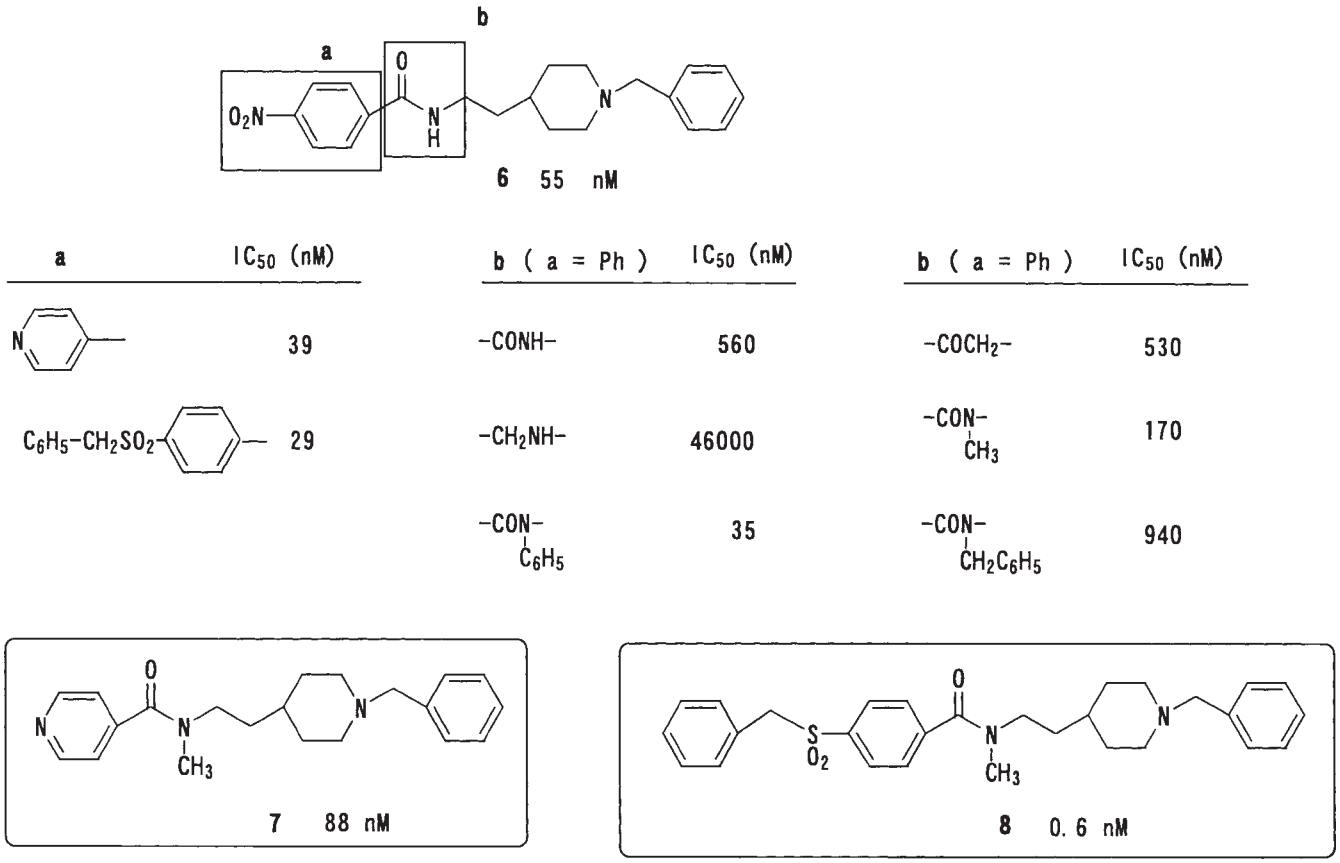

Fig. 2. SAR of benzamide derivatives.

pounds of benzamide derivatives (6). This is a summary of the SAR of benzamide derivatives (Fig. 2). If the phenyl group was replaced with pyridine or the benzylsulfonyl group, the activity was maintained. If the amide group was replaced with a COCH2 group, there was no change in the activity, but when a CH2NH group was used, the activity nearly disappeared. Introduction of the methyl group and the phenyl group to the nitrogen atom of the amide group increased antiAChE activity. We decided to send two candidates for clinical study: Compound 7 had some very small peripheral side effects of the nervous system, and compound **8** showed the highest antiAChE activity among the benzamide derivatives.[10]

We selected four other compounds as clinical candidates (Fig. 3). Compound **9** showed very specific results *in vivo*: an antiamnesia effect. Compound **10** exhibited very strong antiAChE activity *in vitro*. Compounds **11** and **12** showed remarkable separation between the peripheral nerve side effects and the antiamnesia effect *in vivo*. From these six candidate compounds (7, **8**, **9**, **10**, **11**, **12**), we chose compound **8** as the final candidate for clinical study. Figure 4 shows the flow of the drug design from phenyl ether derivative 1 to benzamide derivative 8 (the clinical candidate).[11]

Unfortunately, our excitement was short-lived because we found that this compound has a very poor rate of bioavailability, as well as a short-term of activity, and was therefore not a suitable candidate for clinical testing. The benzylsulfonyl derivative however, has a novel chemical structure and a selective affinity to AChE, which made it a very attractive compound for study. Immediately after making these findings, we started the screening process again.

Our next drug design strategy was the replacement of the amide moiety with the ketone moiety (**14**). This approach maintained the AChE activity of the compound. In addition, this cyclic-amide derivative (**15**) exhibited enhanced inhibitory action. Based on these results, an indanone derivative (**16**) was designed. The resulting AChE activity was moderate, but we were able to achieve a longer term of action. Subsequently, various indanone derivatives were synthesized and tested for antiAChE activity. Among the indanone derivatives that were developed, donepezil proved to be the most balanced compound (Fig. 5).[12]

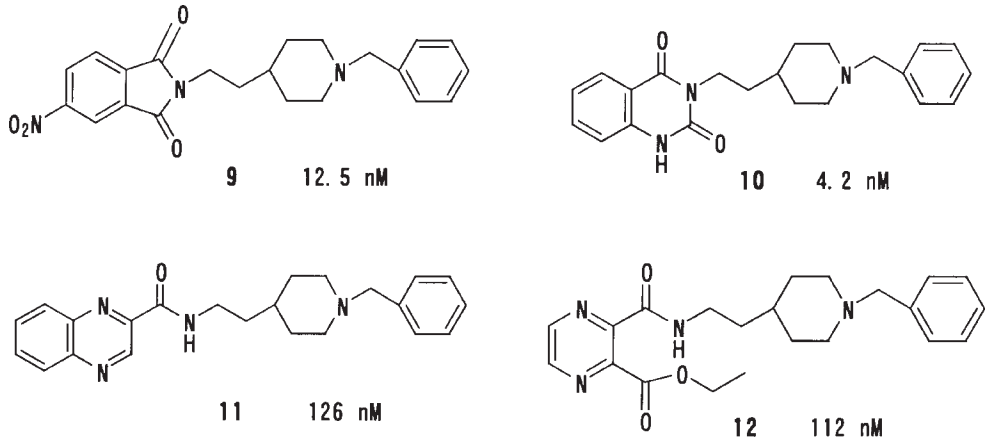

Fig. 3. The first four clinical candidate compounds.

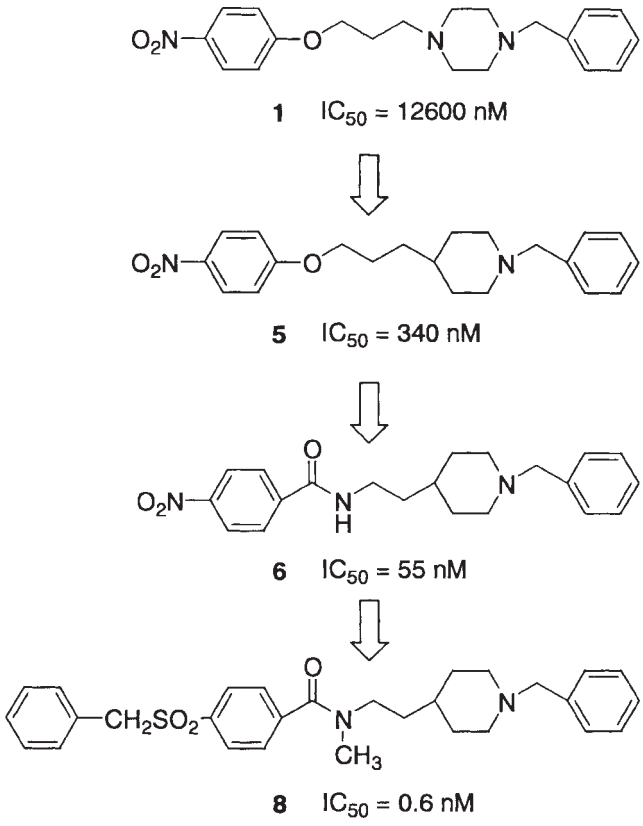

Fig. 4. Flow of drug design to clinical candidate compound **8**.

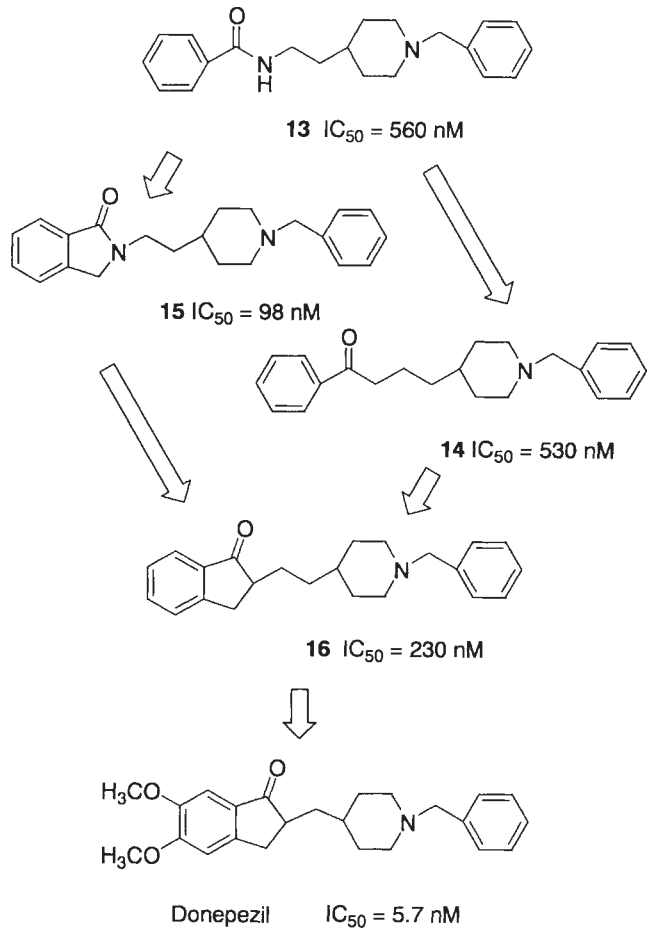

Fig. 5. Flow of drug design to donepezil.

Structure of AChE Complex with Donepezil

Crystal structure

Sussman and coworkers reported the crystal structure of a complex of donepezil with *Torpedo californica* acetylcholinesterase (*Tc*AChE).[13] The atomic coordinates of AChE were obtained from the Brookhaven Protein Data Bank (PDB entry: 1EVE). The X-ray structure, at a resolution of 2.5 Å, showed that the elongated donepezil molecule spans the entire length of the active-site gorge of the enzyme (Fig. 6; green grid, solvent-accessible surface of the active-site cavity; prink balls, water molecules. Space-filling atoms are shown for donepezil. Residues that participate in interactions are purple. Residues involved in the catalytic triad are orange). It thus interacts with both the "anionic" subsite, at the bottom of the gorge, and with the peripheral anionic site, near its entrance, via aromatic stacking interactions with conserved aromatic residues. It does not interact directly with either the catalytic triad or with the "oxyanion hole."

One face of the benzyl benzene ring stacks against the indole ring of Trp-84 at the anionic subsite near the bottom of the gorge in both the model and crystal structure. On the opposite face is a classic aromatic hydrogen bond with a water molecule (WAT1160). This water is held firmly by a hydrogen bond to another water molecule (WAT1161), in the "oxyanion hole," and to WAT1159.

In the constricted region, halfway up the gorge, the charged nitrogen of the piperidine ring makes a cation-π interaction[14] with the phenyl ring of Phe-330. Although docking simulation predicted two more possible interactions between the charged nitrogen of the piperidine ring and the carboxyl group of Asp-72 and the hydroxy group of Tyr-121, the ring nitrogen actually makes an in-line hydrogen bond with WAT1159.

At the top of gorge, the indanone ring stacks against the indole ring of Trp-279 in a parallel π-π interaction. However, none of the binding partner to the methoxy group at the indanone moiety is found in the crystal structure.

Preclinical Pharmacology of Donepezil (E2020)

The following experiments were designed to evaluate the properties of donepezil, a new cholinesterase inhibitor, with respect

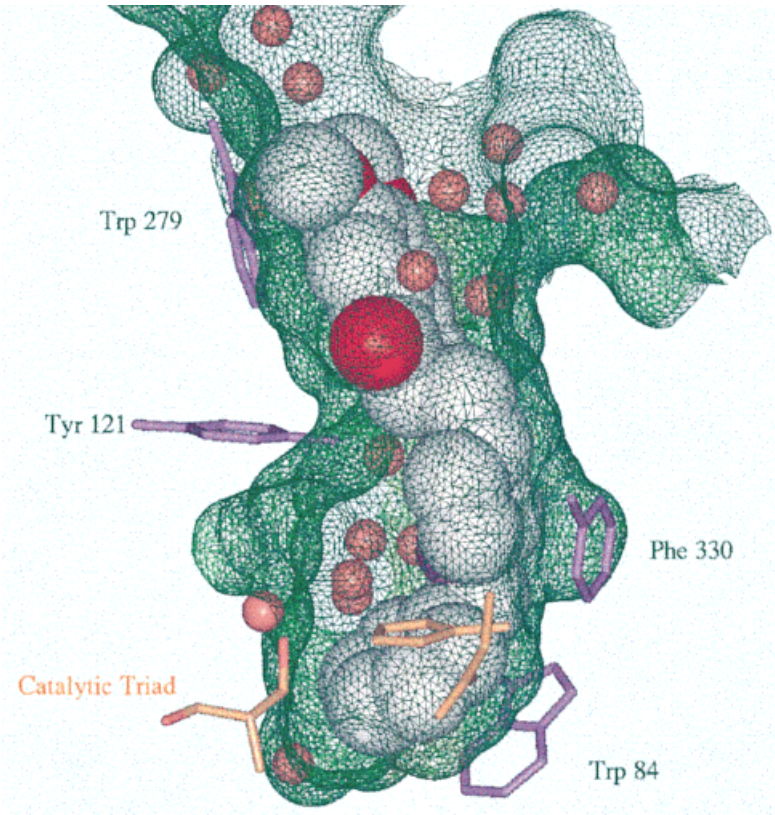

Fig. 6. Active-site cavity of *Tc*AChE with donepezil molecule obtained from the Brookhaven Protein Data Bank (PDB entry: 1EVE).

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

Compound	IC_{50}(n*M*)		Ratio of EC_{50}s′
	AChE activity	BuChE activity	(BuChE/AChE)
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.

to its effect on the central cholinergic system. Conventional ChE inhibitors such as tacrine and physostigmine were used as reference compounds in some experiments.

Effects on cholinesterase 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, physostigmine (PHY) and tacrine. Rat brain homogenates were used as the source of AChE and rat plasma served as the source of BuChE. ACh was used as the substrate for AChE, and butyrylthiocholine (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 listed in Table 1.

Effects of donepezil on brain ACh concentrations

In view of the clear-cut capability of donepezil to inhibit brain cholinesterase activity *in vitro* and *in vivo*, it was reasonable to assume that donepezil could increase the concentration of ACh in the brain. This was tested in two different systems:

1. In the first set of experiments, donepezil was administered orally to rats. The animals were then sacrificed by microwave irradiation of the head 1 h later. The cerebral cortex, hippocampus, and striatum were dissected, and ACh was extracted and measured using an HPLC technique. The results (Table 2) indicate that oral administration of donepezil caused a dose-dependent increase in ACh in all three areas of the brain tested.
2. By using a microdialysis technique, the effect of donepezil on the concentration of ACh in the extracellular space of the rat brain cortex was measured.[15] A microdialysis probe was implanted into the cerebral cortex of conscious rats and perfused with buffer. The ACh that was released into the extracellular space was collected with the perfusing buffer and analyzed by HPLC. Baseline release of ACh was measured for at least 60 min, and the amount of ACh in the perfusate was determined at 20-min intervals. Donepezil was given intraperitoneally at doses of either 1 or 3 mg/kg, and 20-min samples were analyzed over the next 2 or 3 h. The results were expressed as a percentage of the average release measured during the control period (Fig. 7). From this experiment, it is clear that E2020 increases ACh in the extracellular space of the rat cerebral cortex in a time- and dose-dependent manner.

From the preceding experiments, we concluded that donepezil is capable of decreasing AChE activity and increasing the ACh concentration in the cerebral cortex of normal animals.

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

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

In the first study, the neurotoxin ibotenic acid was injected into the *nucleus basalis magnocellularis* region of the rat brain. Destruction of this region, which enervates the cerebral cortex, causes a decrease in the concentration of ACh in the cerebral cortex. Two to three weeks after injection, the animals were

Table 2. Effects of E2020 on ACh concentrations in the cerebral cortex, hippocampus, and striatum of rats.

Treatment	Dose mg/kg	n	ACh concentration (nmole/g)		
			Cortex	Hippocampus	Striatum
E2020	—	6	15.8 ± 0.79	23.5 ± 0.75	67.7 ± 3.52
	5	6	20.4 ± 0.69*	25.6 ± 0.78	87.8 ± 3.99
	20	6	21.8 ± 1.44**	28.7 ± 1.14**	99.3 ± 8.95**

Figures are mean ± SE. *,**: $p < 0.05$, 0.01, respectively, vs. respective control (Dunnett's *t*-test).

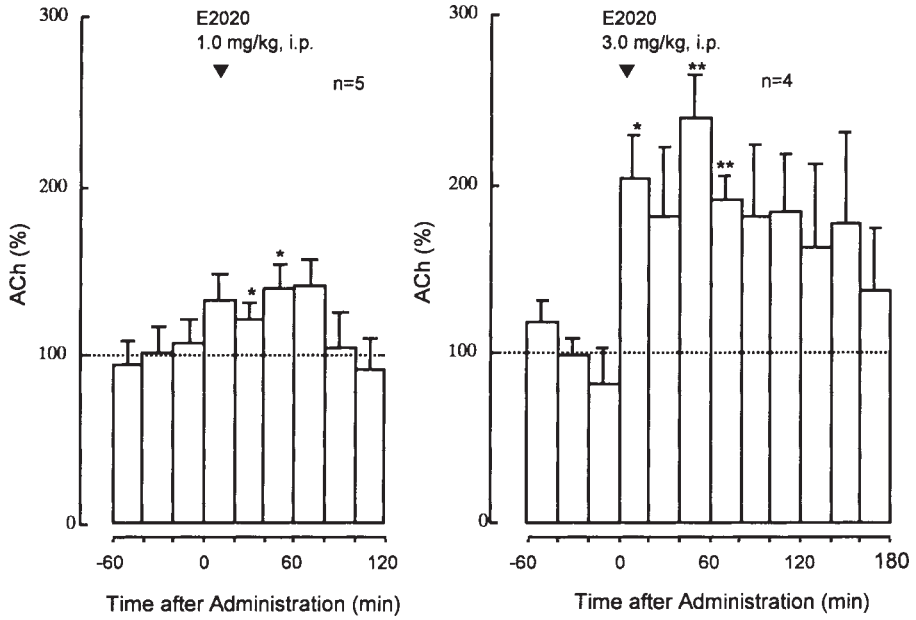

Fig. 7. Effects of E2020 on extracellular ACh levels in the cerebral cortex of rats. *,**: $p < 0.05$, 0.01, respectively, vs. baseline release (paired *t*-test, n = 4–5).

administered an oral dose of either donepezil or tacrine. One h 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. 8) indicate that exposure to ibotenic acid significantly decreases 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), causes a dose-dependent increase in cortical ACh. In this model system, donepezil appears to be a more potent agent than tacrine.

Based on these three studies, it has been established that oral doses of donepezil can increase the concentrations of ACh in the brains of animals with abnormally low levels of ACh.

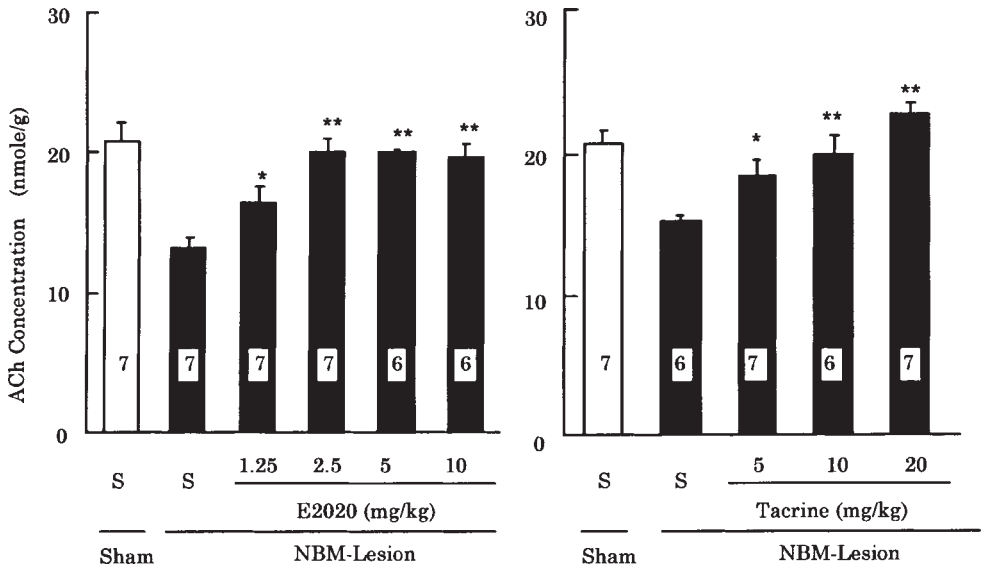

Fig. 8. Effects of E2020 and tacrine on ACh concentration in the cerebral cortex of rats with ibotenic acid-induced NBM lesions. *,**: $p < 0.05$, 0.01, respectively, vs. NBM-lesioned rats (Dunnett's *t*-test). S: Saline; Sham: Sham

Effect of donepezil in behavioral models of cholinergic hypofunction

Based on the previous studies, it is apparent that donepezil is a relatively specific inhibitor of AChE, which can increase the concentration of ACh in both normal and ACh-deficient animals. The final set of studies was designed to determine the ability of donepezil to alter behavior that is impaired due to a deficiency in cortical ACh. Accordingly, donepezil was tested for its effect on several model systems of abnormal animal behavior.

The second 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).[16] The NBM was destroyed in test animals by bilateral injection of ibotenic acid. After 1 week, NBM-lesioned and sham-operated animals were placed in a passive avoidance box consisting of light and dark compartments, where they were trained, using electric shock, to avoid entry into the dark compartment. One h prior to training, they were given either donepezil, tacrine, or saline, orally. After 24 h, they were tested 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 that retained the training, i.e., memory of the electric shock, had longer latency times. As shown in Figure 9, 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 was not statistically significant. These results indicate that donepezil is capable of enhancing the retention of training (memory) in animals with cholinergic hypofunction.

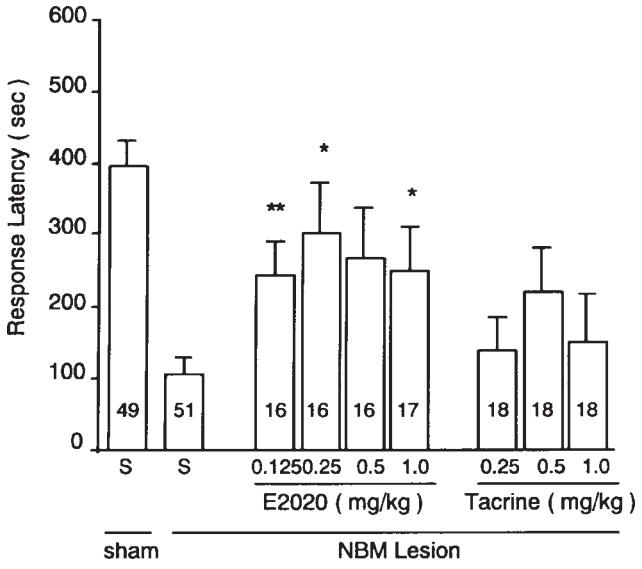

Fig. 9. Effects of E2020 and tacrine on the latency of passive avoidance response in NBM-lesioned rats. *,**: $p < 0.05$, 0.01, respectively (Mann-Whitney's U-test). S: Saline; Sham: Sham-operated rats; NBM Lesion: NBM lesioned rats. The number in each column represents the number of animals.

Clinical Studies of Donepezil

US Multicenter Study Phase II

A double blind, placebo-controlled, randomized trial using doses of 1, 3, and 5 mg/day of donepezil in 141 patients was reported in 1996. A 12-week double-blind phase was followed by a one-week single-blind placebo washout. Improvements in the Alzheimer's Disease Assessment Scale (ADAS-cog) and Mini-Mental State Exam (MMSE) scores were reported; no changes were found in this short-term study 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.

Fifteen-Week Phase III Study

In a phase III study, approximately 150 patients each received either donepezil 5 mg/day, donepezil 10 mg/day, or a placebo once daily for 12 weeks followed by a single-blind placebo washout for 3 weeks.[17] The 10-mg/day dose was titrated using a blind schedule in which subjects received 5-mg doses of donepezil for the first 7 days. Consistent with FDA guidelines, the principal outcomes were measured by ADAS-cog and CIBIC-Plus (Clinician's Interview-Based Impression of Change-Plus). Statistically significant improvements in ADAS-cog scores were seen within 3 weeks continuing to the study endpoint and were significantly different from the placebo group. Significant improvements in this study were also seen in the CIBIC-Plus at both the 5- and 10-mg doses.

Thirty-Week Phase III Study

In this study, which was similar in design to the 15-week study, approximately 150 patients were administered daily doses of donepezil 5 mg, donepezil 10 mg, or a placebo. The patients were followed for 24 weeks, followed by a 6-week washout.[18] Again, there were statistical improvements in the ADAS-cog in patients treated with either dosage at 12 and 18 weeks. CIBIC-Plus scores also improved in both groups in comparison to the placebo group (Figs. 10,11).

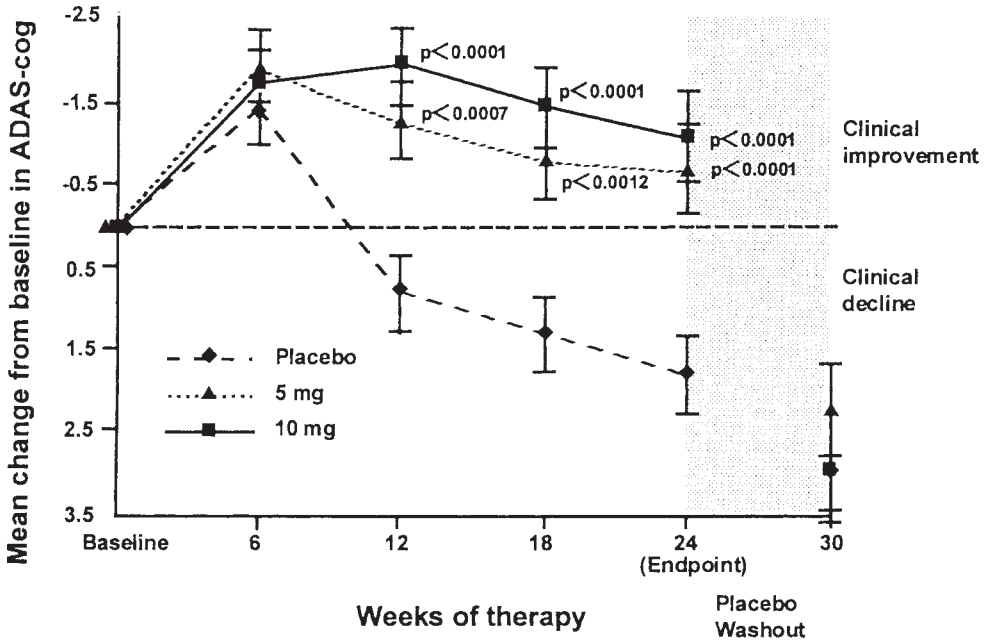

Fig. 10. Effect of E2020 on cognitive function measured using ADAS-cog.

Conclusions

The discovery of donepezil has become a turning point in the development of AChE inhibitor as a treatment drug for Alzheimer's disease. We found the seed compound by chance and, after 4 years of exploratory research, we were able to develop a novel antiAD drug. We are very proud of this discovery particularly because donepezil is the best AChE inhibitor developed so far. A notable characteristic of this compound is its strong antiAChE activity and a very high degree of selectivity

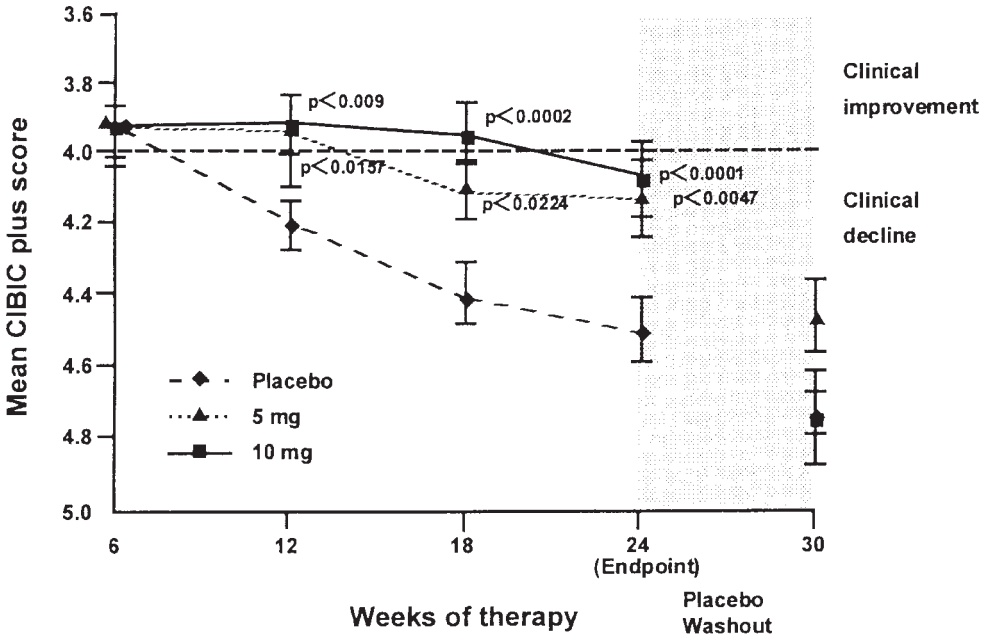

Fig. 11. Effect of E2020 on global function measured using CIBIC-Plus.

for AChE. In studies on both animals and humans, donepezil showed a very long half-life, indicating that it is ideal for the convenient once-daily dosage. The success of donepezil has provided the impetus for the continuous development of AChE inhibitors for the treatment of AD. Other drug developers have pursued the cholinergic hypothesis in AD drug development.

Donepezil (E2020) is now marketed in more than 50 countries worldwide under the trade name Aricept®.

I would like to thank Ms. Mary Alpa Cornelio, of Eisai Co., Ltd., for her valuable assistance in compiling this paper.

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