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REVIEW —*New Drug and Recent Technique*—

Research and Development of Donepezil Hydrochloride, a New Type of Acetylcholinesterase Inhibitor

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ABSTRACT—A wide range of evidence shows that cholinesterase (ChE) inhibitors can interfere with the progression of Alzheimer's disease (AD). The earliest known ChE inhibitors, namely, physostigmine and tacrine, showed modest improvement in the cognitive function of AD 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 (AChE) inhibitor that would overcome the disadvantages of these two compounds. During the study, by chance we found a seed compound. We then conducted a structure-activity relationship study of this compound. After four years of exploratory research, we found donepezil hydrochloride (donepezil). Donepezil showed several positive characteristics including the following: 1) It has a novel structure compared to other conventional ChE inhibitors; 2) It shows strong anti-AChE activity and has long lasting efficacy; 3) The inhibitory characteristic of donepezil shows that it is highly selective for AChE as compared to butyrylcholinesterase (BuChE) and showed reversibility; 4) The results of clinical studies on donepezil show a very high significant difference on ADAS cog and CIBIC plus scores of AD patients. Donepezil is currently marketed in 56 countries all over the world.

Keywords: Donepezil, Alzheimer's disease, Acetylcholinesterase inhibitor, Structure-activity relationship, Placebo controlled study in Japan

1. Introduction

The aged population in the world clearly is increasing. Correlating with this increase, however, is the probability of mental health decline or dementia. Alzheimer's disease (AD) is said to be the leading cause of dementia in elderly individuals. With the continuing increase of the elderly population, the prevalence of AD is likely to increase.

AD individuals exhibit retrogression in mental health functions rendering them incapacitated and unable to perform normal daily activities. Elderly persons are the ones commonly afflicted with this disease. However, evidence shows that it can also afflict even individuals as young as 40 years of age. Ironically, the true nature or cause of the disease is still unknown, making the development of treatment drugs a complex endeavor. Currently, the loss of cholinergic function is the only evidentiary finding responsible for cognitive decline. Hence, therapeutic development has focused on this theory.

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2. Alzheimer's disease

2.1. Definition

Alzheimer's disease, discovered by Dr. Alois Alzheimer in 1907, is described as a degenerative disease of the central nervous system characterized especially by premature senile mental deterioration (1). 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.

2.2. Prevalence

In Japan, the most prevalent type of dementia is caused by cerebrovascular diseases affecting about 42% of the dementia population. AD ranks only second, affecting about 32% of the population (2). In the U.S., however, statistics show that AD is the leading cause of dementia affecting about four million of the U.S. population or 10% of Americans over the age of 65 (3).

2.3. Impact on health care

The annual healthcare costs of AD in the U.S. have been estimated to be as high at $100 billion (4). In Japan, the annual healthcare cost of AD is probably much smaller compared to the U.S. However, with the increasing elderly population in Japan, the number of elderly individuals with AD may also be increasing in frequency. It is therefore inevitable that sooner or later, Japanese health authorities will have to confront increasing healthcare figures related to AD.

In addition to the impact on healthcare budget, there is also the emotional as well as physical stress brought to the family of the AD patient. While relatives and caregivers of elderly AD patients have the will to care for them, family care seems to break down sooner or later. Traditionally, the Japanese have had a unique attitude toward misfortune and burden. As a result, many caregivers in Japan endured the care burden because most have accepted it as their fate. But this traditional attitude is slowly fading as more and more younger caregivers in modern Japanese society are choosing not to undertake home care but opt for institutionalization (5).

3. Cholinergic hypothesis

One of the most consistent and profound changes associated with AD is a deficit in central cholinergic neurotransmission, although the alteration of other neurotransmitter systems is also observed. In the 1970s, three laboratories independently found that the activity of choline acetyltransferase (ChAT), the enzyme synthesizing acetylcholine (ACh), was markedly reduced in the cerebral cortex of AD brain (6 – 8). Perry et al. (9) described that the decrease in activity of ChAT in AD brain was correlated with the severity of cognitive impairment. Then, the loss of the large neurons in the Meynert nucleus in the basal forebrain was reported in AD brain (10). This evidence makes us consider that the central cholinergic system might be involved in cognitive dysfunction of AD patients (11, 12).

Early clinical studies of cholinesterase (ChE) inhibitors aimed to activate the central cholinergic system and alleviate cognitive deficits in AD. ChE breaks down released ACh to choline and acetate in the synaptic clefts. ChE inhibitors prevent the hydrolysis of ACh and result in activating cholinergic transmission. Physostigmine had been first tested in a small group of AD patients (13 – 17). The drug was effective to some degree, while there was a negative result in other study (16). These mixed results were due to the short half-life of physostigmine (15 – 30 min) (18, 19) and, presumably, peripheral side effects. Tacrine is another ChE inhibitor (20, 21) and Summers et al. (22) showed improvement of memory score in six out of twelve AD patients who received oral tacrine. This study spurred the development of cholinergic drugs for AD. Tacrine was re-evaluated by a large-scale, placebo-controlled study (23) and was proved to have improving effects. However, at the same time, tacrine was confirmed to cause elevation of hepatic enzymes, ALT/SGPT (23, 24) (Fig. 1).

Donepezil is a piperidine-class AChE inhibitor, rationally designed specially for AD (25, 26). Donepezil was proven to improve cognitive function of mild to severe moderate AD patients and showed excellent tolerability without hepatotoxicity. The long plasma half-life of donepezil (ca. 70 h) allows for a once-daily dosing regimen (27 – 29). The profile of donepezil clearly represents a significant advantage compared with the conventional ChE inhibitors mentioned above (30, 31).

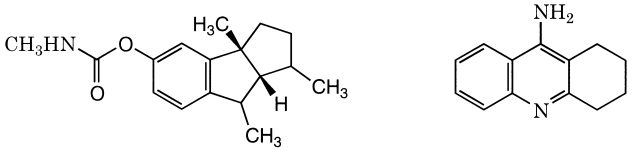
4. Medicinal chemistry of donepezil hydrochloride

4.1. The discovery

Donepezil hydrochloride (E2020, donepezil) is the second drug approved by the U.S. FDA for the treatment of mild to moderate AD. It is a new class of ChE inhibitor having an *N*-benzylpiperidine and an indanone moiety that shows longer and more selective action. It is now marketed in the U.S. and in some European and Asian countries under the trade name of Aricept® (Fig. 2).

The research on donepezil started in 1983. Following research developments on tacrine, our group at Eisai Co., Ltd. started to develop tacrine derivatives. However, we failed to develop a non-toxic tacrine derivative. Through random screening, we encountered an *N*-benzylpiperazine derivative (compound **1**) that was then originally being synthesized in a study on anti-arterial sclerosis. Our tests showed that the anti-AChE activity of the *N*-benzylpiperazine derivative had an IC_{50} of 12.6 μM in rat brain homogenate. This was not very strong but the compound's novel structure was very promising. We decided to use the *N*-benzylpiperazine derivative as the seed compound and synthesized about 700 derivatives (Fig. 3).

Our succeeding experiments showed a dramatic increase



Physostigmine Tacrine

Fig. 1. Physostigmine and tacrine.

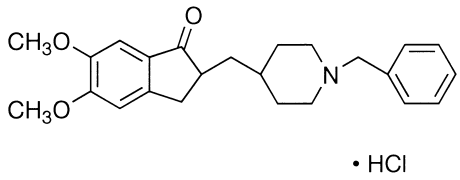


Fig. 2. Donepezil hydrochloride (E2020, Aricept®).

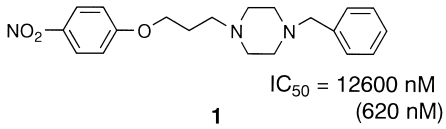


Fig. 3. Structure of seed compound. Reproduced from H. Sugimoto, J. Syn. Org. Chem. Jpn. **56**, 320 – 327 (1998), with permission.

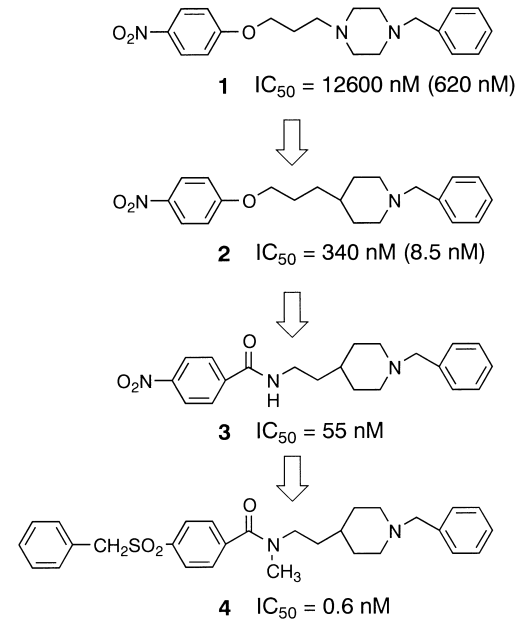


Fig. 4. First stage of drug design. Reproduced from H. Sugimoto, J. Syn. Org. Chem. Jpn. **56**, 320 – 327 (1998), with permission.

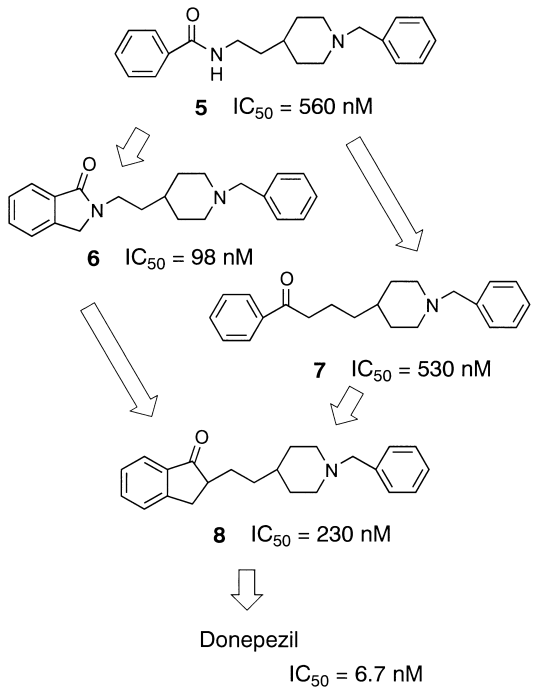


Fig. 5. Second stage of drug design. Reproduced from H. sugimoto, J. Syn. Org. Chem. Jpn. **56**, 320 – 327 (1998), with permission.

in anti-AChE activity when *N*-benzylpiperazine was replaced with *N*-benzylpiperadine. It was a quantum leap in our investigation. Our next challenge was to replace the ether moiety with an amide moiety. This process also increased anti-AChE activity. We thought that the introduction of a functional group at the *para*-position of the benzamide group might increase potency but removal of the nitro group at the *para*-position decreased the potency of the compound.

On the basis of these findings, we synthesized benzamide derivatives. We later on discovered that benzylsulfonyl derivative (compound **4**) was the most potent AChE inhibitor with an anti-AChE activity 21,000-fold greater compared to the seed compound (26, 32, 33) (Fig. 4).

However, our excitement was shortlived 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. However, this benzylsulfonyl derivative has a novel chemical structure and has a selective affinity to AChE, making it a very attractive lead compound. Immediately after these findings, we started the screening process again.

Our next strategy in drug design was the replacement of the amide moiety with a ketone moiety (compound **7**). This approach maintained the AChE activity of the compound. Furthermore, this cyclic-amide derivative (compound **6**) showed enhanced inhibitory action. On the basis of these results, an indanone derivative (compound **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. 5) (26).

4.2. Structure-activity relationships

The indanone derivatives were tested for in vitro inhibition of AChE. A rat brain homogenate was used as the AChE source and the activities were measured according to the method of Ellman et al. (34).

The indanone derivative was divided into four parts as shown in Fig. 6: part 1 (indanone moeity), part 2 (linkage moiety), part 3 (piperidine moiety) and part 4 (benzyl moiety). All data were obtained from the racemic compounds.

Part 1: Table 1 shows the anti-AChE activity of derivatives from the indanone moiety with various bicyclic rings. The effect of replacement of indanone ring with α-tetralone, 1-benzosuberone, 5,6-dimethoxy-1-indanone, 5,6-indanol, 5,6-dimethoxyindene was measured. The ring expansion of cyclic ketone (i.e., compounds **10, 11**) greatly decreased the activity. But the introduction of the methoxy group to the 5,6-position of the indanone moiety increased the activity by 25-fold (donepezil). The carbonyl group of the indanone moiety is essential to the activity since the indanol (compound **12**) and indene (compound **13**) derivatives both showed decreased potency.

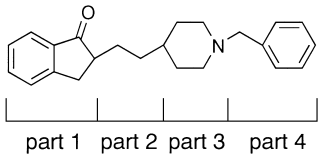


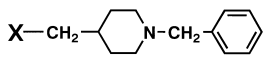
Fig. 6. Four parts of indanone derivatives. Reproduced from H. Sugimoto, J. Syn. Org. Chem. Jpn. **56**, 320 – 327 (1998), with permission.

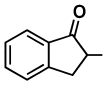
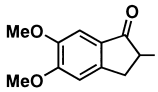
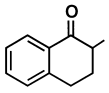
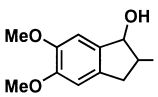
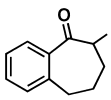
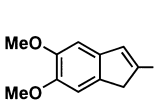
The effect of introducing one or more methoxy groups in the indanone moiety is shown in Table 2. We observed that the introduction of a methoxy group at the R_3-position increased the activity by 20-fold (compound **15**). A methoxy substituent at the R_4-position increased the activity by 10-fold (compound **16**) while substitution at the R_2-position (compound **14**) results in slightly increased activity. These results suggested that the methoxy group at the *para*-position in the carbonyl group of the benzoyl moiety greatly enhanced binding to the active site of the AChE enzyme. Among these derivatives, 5,6-dimethoxyl-indanone derivative or the donepezil, showed the highest activity.

Part 2: Various bridging groups between the indanone moiety and the piperidine moiety were tested. The results are shown in Table 3. Direct connection of the indanone and the piperadine rings dramatically decreased potency (compound **22**). The effect of the length of the bridging moiety on potency varied in the following order: prophylene (compound **26**) > methylene (donepezil) > penthylene (compound **28**) > ethylene (compound **25**) > butylenes (compound **27**). The introduction of an exo-methylene double bond on both the indanone and piperidine moiety decreased the activity (compounds **23, 24**).

Part 3: Table 4 shows the relationships between the location and the number of nitrogen atom and activity. The nitrogen atom at 1-position of the benzylpiperidine moiety was very important since the activity of 4-benzylpiperidine derivative (compound **29**) largely decreased activity. Re-

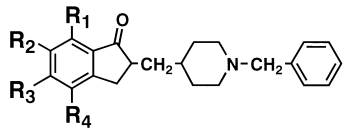
Table 1. Anti-AChE activity of part 1 substituted compounds –1–



Compound no.	X	Inhibition of AChE IC_{50} [nM][a]	Compound no.	X	Inhibition of AChE IC_{50} [nM][a]
9		150	E2020		6.7
10		2100	12		300
11		15000	13		4400

[a] Deviation of measurement of IC_{50} value is 10 – 20%.

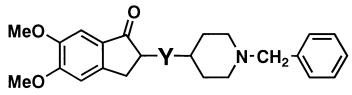
Table 2. Anti-AChE activity of part 1 substituted compounds –2–



Compound no.	R_1	R_2	R_3	R_4	Inhibition of AChE IC_{50} [nM][a]	Compound no.	R_1	R_2	R_3	R_4	Inhibition of AChE IC_{50} [nM][a]
9	H	H	H	H	150	17	OMe	OMe	H	H	85
14	H	OMe	H	H	81	18	OMe	H	OMe	H	25
15	H	H	OMe	H	6.4	19	OMe	H	H	OMe	36
16	H	H	H	OMe	12	20	H	H	OMe	OMe	20
E2020	H	OMe	OMe	H	6.7	21	OMe	OMe	OMe	H	13

[a] Deviation of measurement of IC_{50} value is 10 – 20%.

Table 3. Anti-AChE activity of part 2 substituted compounds



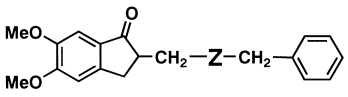
Compound no.	Y	Inhibition of AChE IC_{50} [nM][a]	Compound no.	Y	Inhibition of AChE IC_{50} [nM][a]
E2020	CH_2	6.7	25	CH_2CH_2	30
22	—	3300	26	$CH_2CH_2CH_2$	1.5
23	=CH	13	27	$CH_2CH_2CH_2CH_2$	35
24	CH=	90	28	$CH_2CH_2CH_2CH_2CH_2$	14

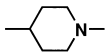
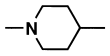
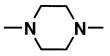
[a] Deviation of measurement of IC_{50} value is 10 – 20%.

placement of the piperadine group with a piperazine group (compound **30**) also resulted in decreased potency. The distance between the carbonyl group in indanone ring and the nitrogen atom in the piperidine ring might be critical for anti-AChE activity.

Part 4: Table 5 shows the relationships between the benzyl moieties. The 3-position-substituted benzyl derivatives showed the highest potency among the 2-, 3- and 4-substituted regioisomers. Substitution of the benzene ring with an electron-donating methyl group and an electron-withdrawing nitro group showed a similar effect. The basicity of the nitrogen atom in the piperidine ring appear to have an important effect in increasing activity since the *N*-benzoylpiperadine derivative (compound **34**) was almost inactive. Removal of the benzyl group (compound **38**) caused a great reduction in the potency of the compound but the activity was retained after replacement with cyclohexylmethyl group (compound **39**). The replacement of the benzyl moiety with a phenethyl (compound **40**) and 2-napthyl group (compound **41**) decreased potency. Among the indanone derivatives, donepezil is one of the most potent compounds in terms of anti-AChE activity.

Table 4. Anti-AChE activity of part 3 substituted compounds



Compound no.	Z	Inhibition of AChE IC_{50} [nM][a]
E2020		6.7
29		480
30		94

[a] Deviation of measurement of IC_{50} value is 10 – 20%.

5. Pharmacological characteristics

5.1. Inhibition of cholinesterase in vitro

Based on substrate specificity, the ChEs are subdivided into AChE and butyrylcholinesterase (BuChE) (35). AChE is most apt to exhibit synaptic localization and has a role of ACh hydrolysis, while BuChE exists mainly in the glial cells in the brain and its function remains obscure (36).

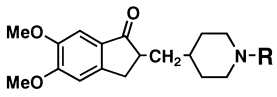
Table 6. Inhibitory effects of cholinesterase inhibitors on rat brain acetylcholinesterase (AChE) and rat plasma butyrylcholinesterase (BuChE)

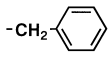
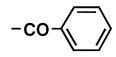
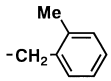
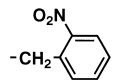
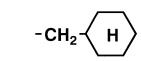
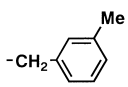
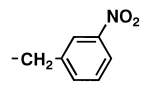
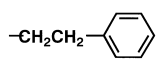
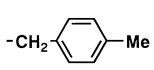
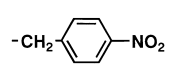
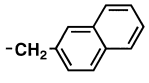
	IC_{50} (nM)	
Drug	AChE	BuChE
Donepezil	6.7 ± 0.35	7400 ± 130
Tacrine	77 ± 1.4	69 ± 1.4
Physostigmine	0.67 ± 0.015	16 ± 0.65
Rivastigmine	4.3 ± 0.087	31 ± 2.0

Values represent the mean ± S.E.M. from 4 dose-response curves for each test drug.

Donepezil highly and selectively inhibits AChE (IC_{50}: 6.7 nM) more than BuChE (IC_{50}: 7.4 μM) (Table 6) (37). On the contrary, tacrine inhibits the both enzymes by the same degree (AChE, IC_{50}: 77 nM; BuChE, IC_{50}: 69 nM). This selectivity of donepezil toward AChE depends on its chemical structure, and most piperidine-based cholinesterase inhibitors showed high selectivity (25). Carbamate ChE inhibitors like physostigmine and rivastigmine need preincubation before showing full inhibition on ChEs in vitro. We reevaluated the inhibitory potency of physostigmine and rivastigmine precisely on AChE and BuChE under a suitable condition of preincubation (37). This study confirmed that physostigmine and rivastigmine have mild selectivity toward AChE compared with BuChE. Donepezil almost equally inhibits AChEs of electrical eels (38), rat brain homogenates (37) and human erythrocytes (38, 39),

Table 5. Anti-AChE activity of part 4 substituted compounds



Compound no.	R	Inhibition of AChE IC_{50} [nM][a]	Compound no.	R	Inhibition of AChE IC_{50} [nM][a]	Compound no.	R	Inhibition of AChE IC_{50} [nM][a]
E2020		6.7	34		>10000	38	H	5400
31		10	35		160	39		8.9
32		2.0	36		4.0	40		180
33		40	37		100	41		2900

[a] Deviation of measurement of IC_{50} value is 10 – 20%.

suggesting that the inhibition of AChE by donepezil does not seem to depend on the source of the enzyme used. On the contrary, donepezil, like other ChE inhibitors, had poorer inhibitory activity against AChE solubilized for senile plaques (40), suggesting that AChE in the senile plaques has different characteristics from normal AChE. The mode of AChE inhibition by donepezil is primarily noncompetitive, but donepezil also has a small element of competitive characteristics (41, 42). The inhibition mode of donepezil is, therefore, noncompetitive on the whole, but precisely mixed. The reversibility of AChE inhibition of donepezil is confirmed by comparing its ability to inhibit AChE activity after and before dialysis. Inhibition of AChE by donepezil was recovered by washing out, while dialysis did not affect the inhibition of diisopropylfluorophosphate, a known irreversible inhibitor.

5.2. Neurochemical pharmacology in vivo

Oral administration of donepezil inhibits brain AChE in a dose-dependent manner (Fig. 7A). The minimal effective dose of donepezil to inhibit brain AChE is 0.625 mg/kg, and the ID_{50} value of oral donepezil, the estimated dose to inhibit 50% of brain AChE, is 2.6 mg/kg, while the ID_{50} value of tacrine is 9.5 mg/kg (43). Donepezil is suggested to be three to four times more potent to inhibit brain AChE than tacrine. Furthermore, donepezil inhibits brain AChE in aged rats (26-month-old) as well as in young rats (44). On the contrary, the action of donepezil to inhibit plasma AChE is weak and more than 5 mg/kg is needed to cause significant inhibition. The ID_{50} value of donepezil to inhibit plasma AChE is 37 mg/kg, which is estimated to be about a 14 times higher dosage than that for the inhibition of brain AChE (43). Donepezil shows fairly good permeability to the brain and the concentration of donepezil in the brain is about 6 to 7 times higher than that in plasma. The favorable permeability to the brain supports the central action of donepezil (44) and donepezil is named as a centrally-acting inhibitor. Donepezil did not inhibit ChE in the heart and small intestine and slightly inhibits ChE in pectoral muscles at the dose showing potent inhibition of the brain AChE. Tacrine and physostigmine, on the contrary, inhibit equally both the brain and peripheral tissues like the heart and small intestine (44). These tissue-selective features of ChE inhibition totally depend on selectivity of inhibition on AChE versus BuChE. The dominant type of ChEs in the brain is AChE, while BuChE is superior in peripheral tissues.

Donepezil raises ACh content in hypocholinerigc rat models. Scopolamine, a muscarinic receptor blocker, blocks postsynaptic muscarinic receptor and also facilitates ACh release from cholinergic nerve terminals by blocking presynaptic receptors. This facilitated ACh release causes depletion of the ACh store in the nerve terminals. Donepezil administration counteracts this ACh depletion induced by scopolamine (24). In rats, the cerebral cortex receives the innervation of cholinergic neurons from the nucleus basalis magnocellularis (NBM), which is an analogue of the nucleus basalis of Meynert in humans. Brain lesion of NBM reduces ACh content in the cerebral cortex. Done-

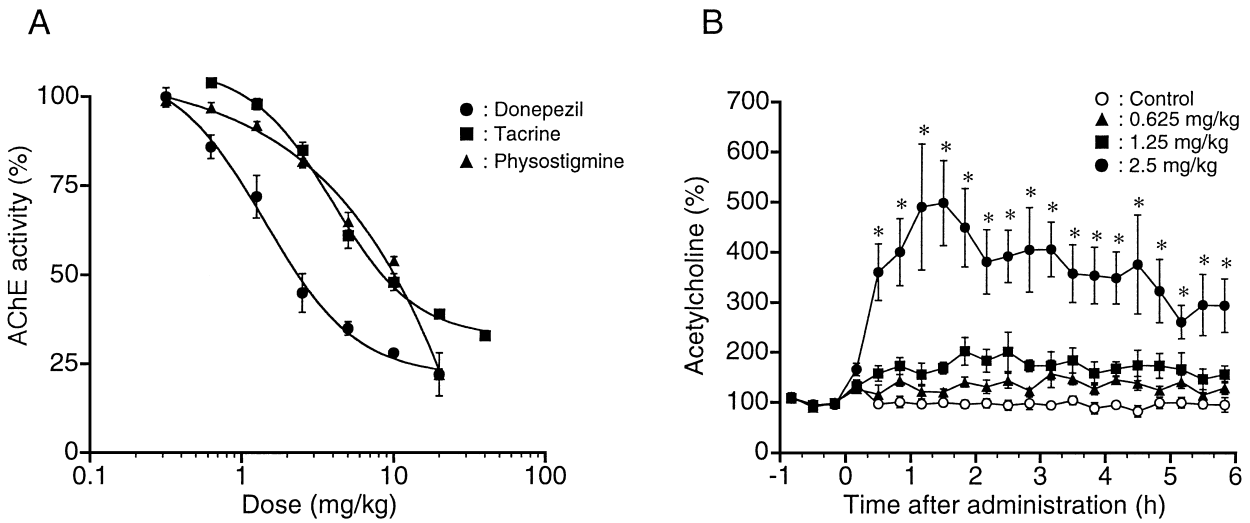


Fig. 7. Neurochemical effects of oral administrations of donepezil on central cholinergic system in rats. (A) Effects of donepezil, tacrine and physostigmine on brain cholinesterase activity in rats. Values represent the mean ± S.E., n = 5. (B) Effects of donepezil on the basal concentration of extracellular acetylcholine in the hippocampus of rats. Data are expressed as a percentage of the pre-levels (average of three samples prior to administration = 100%). Values are means ± S.E., n = 6. Pre-levels were as follows: control: 102.4 ± 18.77, 0.625 mg/kg: 78.3 ± 6.98, 1.25 mg/kg: 92.4 ± 15.20, 2.5 mg/kg: 72.4 ± 6.89 fmol/tube. *P<0.05 vs control (Dunnett's multiple comparison test). Reproduced from H. Ogura et al., Folia Pharmacol. Jpn. **115**, 45 – 51 (2000), with permission.

pezil dose-dependently increases ACh content in the cerebral cortex in rats (24).

Extracellular ACh level in the brain is increased following donepezil administration as revealed by in vivo microdialysis studies (Fig. 7B). Donepezil raised the ACh level in the cerebral cortex and the hippocampus in rats at the dose level of 2.5 mg/kg (45 – 48). The time-course of these increasing effects correlated with ex vivo inhibition of donepezil on brain AChE. It suggests that the increase in extracellular ACh level is based on its inhibitory effects on AChE. Although tacrine also increased extracellular ACh level, the potency of tacrine was about one fourth that of donepezil comparing the effective doses in this study.

5.3. Behavioral pharmacology

Donepezil improves various kinds of learning impairment in hypocholinergic animal models (Fig. 8). The cerebral cortex is one of the most pathologically affected areas in the brain of AD patients. The site receives cholinergic innervation from the Meynert nucleus, where loss of large neurons is frequently observed in AD brain (9). Therefore, the lesion of NBM, which is equivalent to the Meynert nucleus in humans, causes functional disorder of the cerebral cholinergic system and various behavioral alterations. In the step-through passive avoidance response, rats first easily move into the dark compartment of the test box from the light compartment and receive foot shock in the training trial. Then the rats remained in the light compartment to avoid foot shock in the retention trial, if they learn this task. Rats with the lesion of NBM are incapable of remaining a long time in the light compartment in the retention trial. Donepezil administered before the training trial counteracted the effect of NBM lesion on shortening the latency in the retention trial at the dose of 0.125 to 1.0 mg/kg, p.o. without affecting the latency in the training trial (49). Tacrine showed only the tendency to improve the impairment in this study.

In the brain of AD patients, the hippocampus, which is thought to play a crucial role in mnemonic function, is the site where neurofibrally tangles preferentially appear from the early stage of AD (50). The hippocampus receives the cholinergic projection from the medial septum. The lesion of the medial septum results in cholinergic compromise of the hippocampus and impairs spatial learning in rats. Donepezil was administered orally daily to rats that received the lesion of the medial septum during the acquisition of the hidden platform task in the water maze. Donepezil improved the deterioration of learning in this task at the dose of 0.5 mg/kg, while tacrine did not show significant effects in this lesion model (49).

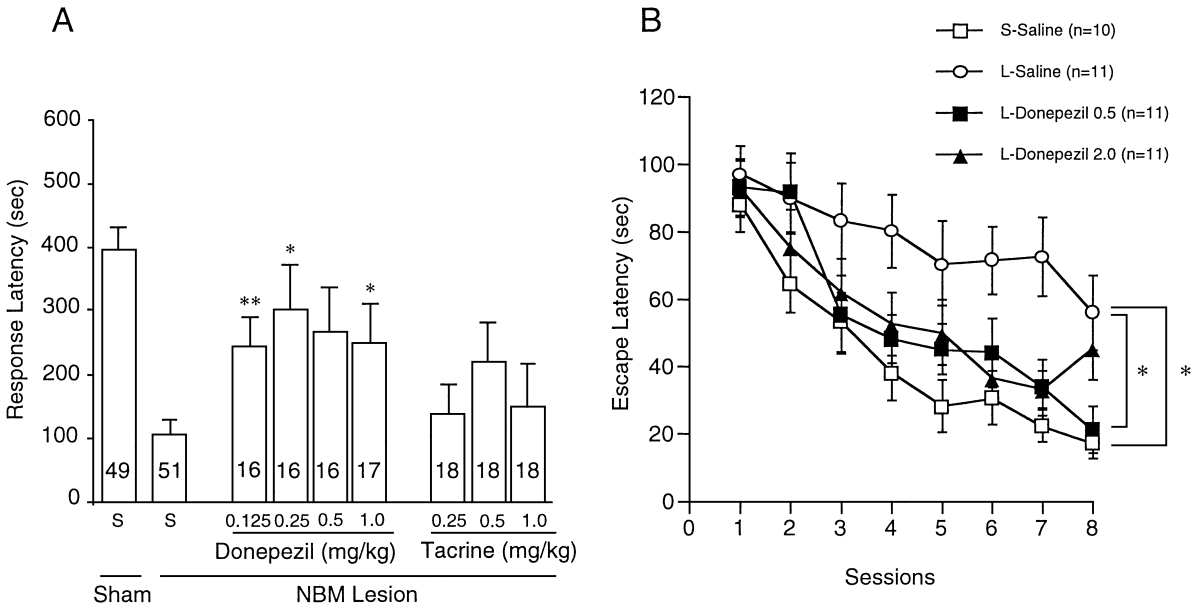


Fig. 8. Improving effects of oral administration of donepezil on impairment of learning in rats. (A) Effects of donepezil on shortening of response latency in passive avoidance test induced by the lesion of nucleus basalis magnocellularis (NBM) in rats. Numbers of rats used are shown by the numbers in the columns. *P<0.05, **P<0.01 (Mann-Whitney U-test). (B) Effects of donepezil on water maze acquisition task in rats with medial septal lesions. Statistical analyses were done using repeated measures of ANOVA followed by the Dunn type multiple comparison test. *P<0.05. S-Saline: Sham-operated saline-treated group, L-Saline: Quisqualate-lesioned saline-treated group, L-Donepezil 0.5: Quisqualate-lesioned donepezil (at 0.5 mg/kg)-treated group, L-Donepezil 2.0: Quisqualate-lesioned donepezil (at 2.0 mg/kg)-treated group. Reproduced from H. Ogura et al., Folia Pharmacol. Jpn. **115**, 45 – 51 (2000), with permission.

Scopolamine, an anti-cholinergic, causes amnesia in humans and also impairs learning in animals. We administered scopolamine to rats that were well trained to obtain pellets in the 8-arm radial maze. The agent induced an increase in errors and total running time. Donepezil alleviates the increasing errors and running time at 0.5 mg/kg, while a 4 times higher dose of tacrine (2 mg/kg) was needed (49). The same improving effect of donepezil on scopolamine-induced deficit in the radial maze task was reported by other researchers (51, 52), who used donepezil as a reference drug to evaluate their compounds. In other scopolamine-induced models, donepezil partially, but significantly counteracted scopolamine-induced delayed-matching-to-position task in rats (53, 54). Rupniak et al. (55) showed that donepezil inhibited scopolamine-induced deficit in spatial and visual recognition memory tasks in rhesus monkeys. These results suggest that the effect of donepezil on scopolamine-induced deficits does not seem to depend on the types of learning tasks or animals used.

Preference of action to central, rather than peripheral, cholinergic system is critical for ChE inhibitors as a therapy for AD. The proportion of central versus peripheral cholinergic activity of a number of ChE inhibitors was clarified by observing yawning and fasciculation as a marker of central and peripheral cholinergic sign, respectively (56). Donepezil was found to potently and preferentially activate the central cholinergic system in rats, while having relatively little effect on the intensity of fasciculation, in comparison with tacrine and physostigmine. Dronfield et al. (57) also demonstrated that donepezil had more selectivity than tacrine for central (tremor) versus peripheral (salivation/lacrimation) effects in rats. Difference between effective doses in behavioral and neurochemical studies and dose causing fasciculation indicates the safety margin. The ChE inhibitors were compared, and donepezil was found to have a relatively wide safety margin compared to the other ChE inhibitors (46, 49).

5.4. Conclusive remarks of pharmacological studies

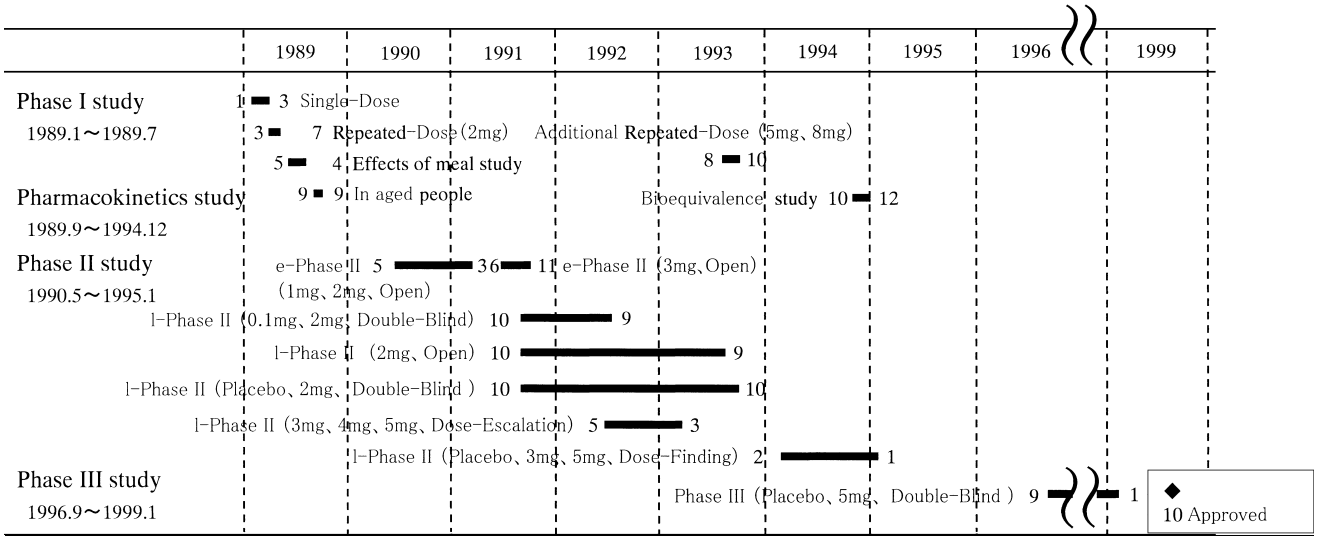
The neurochemical studies show that donepezil is a highly selective, reversible and noncompetitve inhibitor of AChE in vitro and the inhibitory effect of donepezil is relatively selective for brain ChE, i.e., AChE in vivo. Donepezil is capable of increasing ACh not only in the brain of normal rats and in the extracellular space of rat cerebral cortex, but also in the rat models of cholinergic hypofunction. In the systems used for these studies, donepezil was a more selective inhibitor of brain AChE than the reference compound tacrine. In the behavioral studies, donepezil improves various kinds of learning impairment in hypocholinergic rat models. Comparison between central and peripheral cholinergic activation among some ChE inhibitors revealed that donepezil preferentially activates the central cholinergic system compared with the peripheral cholinergic system. These results suggest that donepezil is a potent centrally-acting ChE inhibitor and suitable for AD therapy.

6. Clinical study in Japan

6.1. Background of clinical study

Various ChE inhibitors have been studied as drugs to

Table 7. Course of clinical trial



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treat Alzheimer's disease, but in Octber 1999, donepazil was approved as the first drug in Japan to treat this disease. This drug was the subject of 13 clinical trials in total over a period of 10 years starting with a Phase I study in 1989 (Table 7). In the first trials, clear efficacy was not observed, but in the Phase III clinical trial with two primary endpoints (ADAS-J cog, J-CGIC), better efficacy than with the placebo was achieved. This part of the review will introduce the results of the Phase III study in Japan, which was the final key to obtaining approval.

6.2. Phase III clinical study

1) Preparation of the protocol

As shown in Table 7, there was a preparatory period of about one year and 6 months from completion of the dose finding study until the start of the Phase III study. This delay was caused by the fact that the entire protocol had to be completely revised because the initial objective was not achieved in the dose finding study, and it was necessary to wait for completion of the two pivotal studies in the U.S. and reflect these results in the protocol. In the Phase III study protocol, these findings and the basic stance of the antidementia drug guidelines in preparation were incorporated as much as possible.

2) Outline of the study method

This study was a 24-week placebo-controlled double-blind comparative study on patients with mild to moderate AD performed in 54 facilities in Japan. The subjects were selected from among outpatients with mild to moderate AD diagnosed using the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV) with Mini-Mental State Examination (MMSE) scores of 10 to 26 and Japanese version of the Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-J cog) scores of 15 or higher. Imaging diagnosis by CT or MRI was also always used for the diagnosis, and the absence of localized cerebral lesions or multiple infarcts, which could be considered as a cause of the dementia, was confirmed. The Hachinski ischemia score was used to differentiate from cerebrovascular dementia. Concomitant medication was prohibited as a rule, and the eligibility of the patients was verified twice at screening and immediately before administration by establishing an observation period before administration.

The efficacy of donepezil was evaluated by superiority over the placebo in changes in the ADAS-J cog score and the global clinical symptom evaluation [Japanese version of the Clinical Global Impression of Change (J-CGIC)]. Dual assessment of the primary endpoints was performed since it is recommended in the FDA guidelines. Therefore, when there was no significant difference from the placebo in either assessment, the efficacy of donepezil was not considered to be verified by this study.

Some explanation is required concerning the J-CGIC, which was set as one of the primary endpoints of efficacy. This is given below: The J-CGIC is an evaluation method by which the global changes of patients are evaluated subjectively by clinicians into seven grades: 1. markedly improved, 2. improved, 3. slightly improved, 4. no change, 5. slightly aggravated, 6. aggravated, 7. markedly aggravated. It is the same as the CGIC used for the dose finding study in the U.S. In the U.S., the Clinician's Interview-Based Impression of Change (CIBIC) (58) and AD Cooperative Study – Clinical Global Impression of Change (ADCS – CGIG) (59) have been developed with the objective of supplementing the low reliability of the CGIC. However, these systems do no suit actual conditions in Japan because clinicians other than the attending physicians must make evaluations based on interviews with the patients before and after treatment. No Japanese versions have been prepared (at present, a Japanese version is being prepared as CIBIC plus-J (60) and they are difficult to use. Therefore, in this study, the basic structure of the "Global Improvement Evaluation" used to date was revised and the problems were solved by adding the structural factors of the evaluation methods that were lacking: 1) appropriate name, 2) clear definition for the phenomena involved, and 3) clear indication of the anchor points at each stage.

In addition, the Clinical Dementia Rating-Sum of the Boxes (CDR-SB), Mental Function Impairment Scale (MENFIS) and the Caregiver-rated Modified Crichton Scale (CMCS) have been established as auxiliary efficacy evaluation scales and were evaluated every 4 weeks after treatment.

3) Study results

There were 268 patients enrolled in this study, and 39 patients (15%) were discontinuations or dropouts during the study. The main reason for the discontinuations or dropouts was a request for discontinuation from the patient or family members in 10 cases. This was followed by adverse reactions, and complications or adventitious diseases in eight cases each. The mean age of the patients was 70.1 years (range: 52 to 83) in the donepezil group and 69.4 years (range: 48 to 90) in the placebo group. The gender ratio (male:female) was 32%:68% in the donepezil group and 34%:66% in the placebo group (Table 8).

An imbalance in patient characteristics between the administration groups was found in the preadministration MMSE and ADAS-J cog score, but when an analysis of covariance (ANCOVA) and the Cochran-Mantel-Haenszel (CMH) test adjusted for the imbalance in patient characteristics were performed, it was confirmed that there was no effect on interpretation of the results of this study.

Figure 9 shows changes in the ADAS-J cog score as the primary efficacy endpoint in this study. The mean level of change of the ADAS-J cog score from baseline was

Table 8. Patient characteristics

Item		Donepezil, 5 mg/day n = 116	Placebo n = 112	*p* value[#]
Sex, n (%)	male	37 (32)	38 (34)	0.853
	Female	79 (68)	74 (66)	
Age (Mean ± SD)		70.1 ± 7.6	69.4 ± 8.8	0.521
Range		52 – 83	48 – 90	
Weight, kg (Mean ± SD)		51.3 ± 8.4	50.0 ± 9.3	0.316
Range		33 – 70	29 – 73	
Severity (CDR)	CDR 1	79 (68)	69 (62)	0.305
n (%)	CDR 2	37 (32)	43 (38)	
MMSE score (Mean ± SD)		17.8 ± 3.9	16.6 ± 3.9	0.035*
Range		10 – 26	10 – 26	
ADAS-J cog score (Mean ± SD)		22.91 ± 8.49	26.90 ± 9.84	0.001*
Range		15.0 – 56.7	15.0 – 60.0	

[#]: Using U test except for Sex (x^2 test), *: $p<0.15$. Modified from Y. Arai, Rinshouseishinyakuri **3**, 1019 – 1025 (2000), with permission.

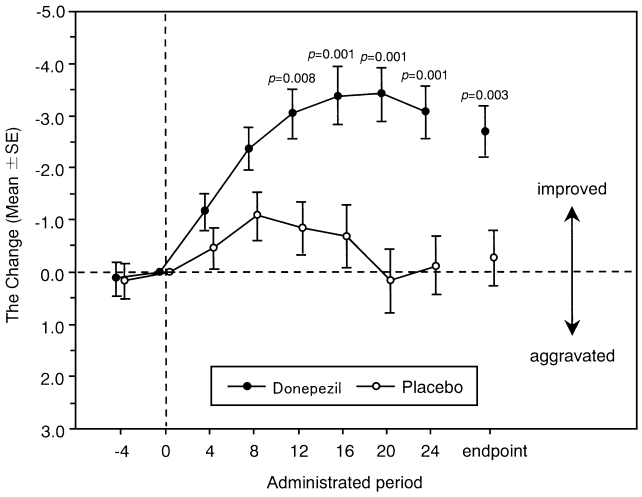

Fig. 9. Baseline change in ADAS-J cog. Modified from Y. Arai, Rinshouseishinyakuri **3**, 1019 – 1025 (2000), with permission.

−2.70 in the donepezil group and −0.26 in the placebo group. The difference in the mean intergroup levels of changes (treatment group – placebo group) was −2.44, and the donepezil group showed significant improvement with respect to the placebo group ($P = 0.003$, *U*-test).

Figure 10 shows the results of J-CGIC, one of the primary endpoints. The improvement rate (percentage of slightly improved or better) in the donepezil and placebo groups was 52% and 22%, respectively, and the worsening rate (percentage of slightly aggravated or worse) was 17% and 43%, respectively. Efficacy with respect to the global clinical symptoms was confirmed in the donepezil group ($P<0.001$, *U*-test).

The efficacy of donepezil was also confirmed for the other secondary efficacy endpoints. Confirmation of the efficacy of donepezil by the two primary endpoints and the other secondary endpoints suggested that the objective

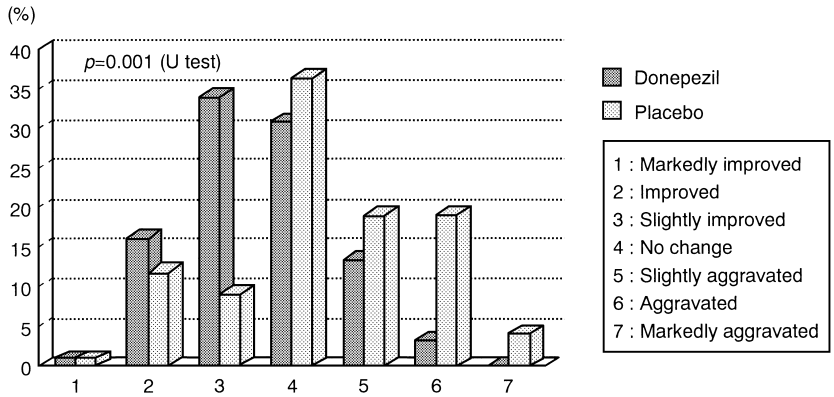

Fig. 10. Result of J-CGIC. Modified from Y. Arai, Rinshouseishinyakuri **3**, 1019 – 1025 (2000), with permission.

of the study was achieved and improvement of the cognitive function of the patients had an effect on the mental function and the activities of daily living. The improvement of cognitive function according to ADAS-J cog obtained in this study was similar to the results obtained in the U.S. study in terms of the difference in level of change from the placebo (treatment group – placebo group) (28) (this study: –2.54, U.S.: –2.49). Reproducibility of these trial results was also confirmed.

The incidence of adverse events was 40% (54/136) in the donepezil group and 25% (33/131) in the placebo group. The incidence was significantly higher in the donepezil group ($P = 0.016$, Fisher's exact test). However, the incidence of adverse reactions for which a causal relation with administration of donepezil could not be ruled out was 10% (14/136) in the donepezil group and 8% (10/131) in the placebo group. There was no significant difference between the groups ($P = 0.587$, Fisher's exact test). The main adverse reactions in the donepezil group were gastrointestinal disorders including three cases of diarrhea (2%), three cases of nausea (2%), two cases of constipation (1%), one case of abdominal pain (1%), one case of vomiting (1%), one case of anorexia (1%) and one case of abdominal fullness (1%). These gastrointestinal adverse reactions were mild to moderate and they all disappeared during continued administration or after temporary withdrawal of the drug.

6.3. Conclusive remarks of clinical study in Japan

The clinical trials included a 52-week open study (61) to confirm the safety of long-term administration, and a 24-week open study (62) using a fine granular formulation to facilitate ingestion of the drug, which was performed in parallel with the Phase III study. The objective in each study was to confirm the efficacy and safety of donepezil. After completion of the Phase III study, donepezil was reviewed by the regulatory authorities. As a result, it was approved and licensed for manufacture as Aricept®, the first drug for the treatment of AD, and was launched the following month.

In the clinical studies, the objective was to verify the efficacy and safety of the drug itself, and differences in response at the individual patient level were not examined in detail. This point should be clarified in a study using a larger number of patients. The indication for donepezil is "inhibition of the progression of dementia symptoms in mild to moderate AD." Donepezil is for symptomatic treatment but plays a major role since there has been no method of drug treatment for this disease to date.

As one method of treatment until the discovery of therapeutic drugs, assistance is requested from the patients, their family members and health professionals involved in the treatment.

7. Conclusions

Donepezil inaugurates a new type of ChE inhibitor with a new chemical structure compared to other conventional ChE inhibitors. With donepezil hydrochloride, we were able to prove the cholinergic hypothesis. There are actually three approaches to prove the cholinergic hypothesis: 1) the acetylcholine precursor or muscarinic receptor enhancer, 2) ChE inhibitors and 3) acetylcholine agonists. So far, only ChE inhibitors have succeeded in clinical study for the treatment of AD, but why is it that only ChE inhibitors can succeed in the treatment of AD? At the moment, we do not have an answer to this question. However, it is very interesting to think about it since in our discovery stage, we also experimented on the other approaches of cholinergic theory such as precursor, enhancer, inhibitor and agonist. With donepezil, we are very proud to have developed the highest quality and most effective ChE inhibitor in the world. In the U.S. clinical study, donepezil showed a very high significant difference on ADAS cog and CIBIC plus scores of AD patients. Donepezil is currently marketed in more than 50 countries all over the world. Donepezil is now the leading product for AD treatment in the world.

Acknowledgments

We would like to give our special thanks to the late Dr. Kiyomi Yamatsu who passed away in March 2001. Dr. Yamatsu was the supervisor of the Aricept project team. The Aricept project would not have been a success if not for the able guidance of Dr. Yamatsu. We would also like to thank all our colleagues at Eisai Co., Ltd. who in one way or another helped in the research and development of Aricept.

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