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Inhibitory effect of orally administered donepezil hydrochloride (E2020), a novel treatment for Alzheimer's disease, on cholinesterase activity in rats

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Abstract

Donepezil hydrochloride ((±)-2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-indan-1-one monohydrochloride: E2020: donepezil) is a potent and selective acetylcholinesterase inhibitor developed for the treatment of Alzheimer's disease. The present experiments were designed to compare the inhibitory effects of orally administered donepezil and other cholinesterase inhibitors, tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride), (*S*)-*N*-ethyl-3-[(1-dimethyl-amino)ethyl]-*N*-methyl-phenylcarbamate hydrogentartrate (ENA-713, rivastigmine) and 3-[1-(phenylmethyl)-4-piperidinyl]-1-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-8-yl)-1-propanone fumarate (TAK-147), on the cholinesterase activity in the brain and plasma of rats. Moreover, in order to validate the cholinesterase inhibition data, we measured the brain and plasma concentrations of these drugs. Oral administration of donepezil, tacrine, ENA-713 or TAK-147, caused a dose-dependent inhibition of brain and plasma cholinesterase activities. The ID_{50} values of these compounds for brain cholinesterase activity were 6.3, 40.5, 7.2 and 26.8 μmol/kg, respectively. On the other hand, the ID_{50} values for plasma cholinesterase activity were 89.0, >170, 9.7 and 51.2 μmol/kg, respectively. Thus, the ratios of the ID_{50} (plasma/brain) were 14.1, >4.2, 1.3 and 1.9, respectively. Brain and plasma concentrations of donepezil, tacrine and TAK-147 increased dose-dependently. The ratios of the concentrations (brain/plasma) of these compounds were 6.1–8.4 for donepezil, 14.5–54.6 for tacrine and 7.0–20.6 for TAK-147. The values of 50% inhibitory concentration of these drugs in the brain were 0.42, 3.5 and 1.1 nmol/g, respectively. In contrast, the brain and plasma concentrations of ENA-713 at all doses, except the two highest doses, were below the quantification limit. These results suggest that orally administered donepezil satisfactorily penetrates into the brain and inhibits cholinesterase there, and that donepezil is a potent and selective inhibitor of brain cholinesterase in comparison with plasma cholinesterase in vivo. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Donepezil hydrochloride (E2020); Tacrine; Physostigmine; ENA-713; TAK-147; NIK-247; Cholinesterase inhibitor; Cholinesterase

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disease, characterized by deficits in memory and cognitive function. A remarkable dysfunction of the cholinergic system has been observed in several brain regions of patients suffering from Alzheimer's disease (Bowen et al., 1976; Davis and Maloney, 1976; Perry et al., 1977; Whitehouse et al., 1982), and was shown to be correlated with the severity of cognitive impairment (Perry et al., 1978). These pathological findings, in addition to the fact that the cholinergic system plays a role in memory functions (Drachman, 1977), have led to the hypothesis that enhancement of cholinergic neurotransmission with cholinergic agents may ameliorate cognitive impairment in Alzheimer's disease. Although many attempts have been made to reverse the cognitive impairment using cholinergic agents, cholinesterase inhibitors are the only class of drugs currently approved for the treatment of Alzheimer's disease.

We have developed a novel, piperidine-based, acetylcholinesterase inhibitor, (±)-2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-indan-1-one monohydrochloride (donepezil hydrochloride: donepezil: E2020), for the treatment of Alzheimer's disease (Sugimoto et al., 1995). Donepezil was approved by the US Food and Drug Ad-

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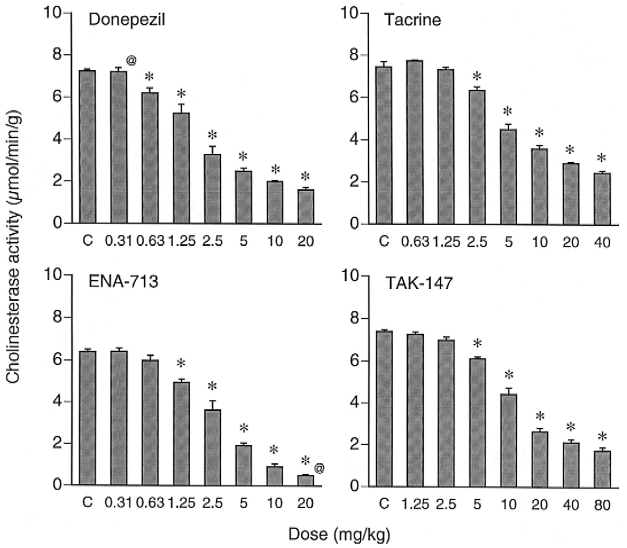

Fig. 1. Inhibitory effects of donepezil, tacrine, ENA-713 and TAK-147 on brain cholinesterase activity in rats. Values represent the mean ± SE. $n = 5$ (@: $n = 4$). * $P < 0.05$ vs. respective control (Dunnett's multiple comparison test).

ministration (FDA) in 1996, and is now being prescribed worldwide. A large-scale multicenter, double-blind clinical study has demonstrated that donepezil is a well-tolerated drug that improves cognitive performance and global function in patients with mild to moderate Alzheimer's disease (Rogers and Friedhoff, 1998; Rogers et al., 1996, 1998a,b). A summary of the preclinical pharmacological data on donepezil has been published (Rogers et al., 1991), describing the effect of oral administration of donepezil on cholinesterase activity in the brain and some peripheral tissues, in comparison with those of tacrine and physostigmine. Donepezil showed more potent and brain-specific cholinesterase inhibition than tacrine or physostigmine. However, there has not been a detailed comparative pharmacological study of the drug, and a complete dose–response curve for brain cholinesterase inhibition has not been obtained.

Recently, many cholinesterase inhibitors have been developed for the treatment of Alzheimer's disease, and some are under clinical trial or have been submitted for approval. (*S*)-*N*-ethyl-3-[(1-dimethyl-amino)ethyl]-*N*-methyl-phenylcarbamate hydrogentartrate (ENA-713: rivastigmine) is a pseudo-irreversible, carbamate-based, cholinesterase inhibitor (Weinstock et al., 1994; Anand and Gharabawi, 1996) and is currently available in some European countries. 3-[1-(Phenylmethyl)-4-piperidinyl]-1-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-8-yl)-1-propanone fumarate (TAK-147) is a piperidine derivative (Miyamoto et al., 1996; Hirai et al., 1997) under clinical study in Japan. Although many pharmacological studies of these cholinesterase inhibitors have been published, the relation between the pharmacological effect of cholinesterase inhibitors and drug concentration in the brain has not yet been examined.

Two types of cholinesterase, acetylcholinesterase and butyrylcholinesterase, are present in a wide variety of tissues. Acetylcholinesterase hydrolyzes acetylcholine, thereby terminating the effect of this neurotransmitter at cholinergic synapses; however, the physiological function of butyrylcholinesterase remains to be established (Massoulié et al., 1993). Cholinesterase in the brain is predominantly acetylcholinesterase (Edwards and Brimijoin, 1982), and is the target of cholinesterase inhibitors used for the cholinergic therapy of Alzheimer's disease. In contrast, peripheral tissues contain both acetylcholinesterase and butyrylcholinesterase, although the proportions of the two enzymes vary from tissue to tissue (Edwards and Brimijoin, 1982). Neither of the enzymes in peripheral tissues is a target for treatment of Alzheimer's disease; rather, their inhibition may cause side effects. In rats, plasma contains approximately equal amounts of acetylcholinesterase and butyrylcholinesterase (Traina and Serpietri, 1984), and therefore, we considered that the inhibitory effect of cholinesterase inhibitors on total cholinesterase activity in rat plasma might reflect their overall influence on peripheral tissues.

In the present study, we measured total cholinesterase activity and obtained complete dose–response curves for brain and plasma cholinesterase inhibition by donepezil, in comparison with tacrine, ENA-713 and TAK-147. Furthermore, in order to examine the relation between the cholinesterase inhibition and drug concentration in the brain and plasma after oral administration of these drugs, we measured the brain and plasma concentrations of these drugs in the same animals in which cholinesterase inhibition was examined.

2. Materials and methods

2.1. Animals

Male Wistar rats (7 weeks of age, 218–276 g, Charles River Japan, Kanagawa, Japan) were housed at a room

Table 1
The ID_{50} values of donepezil, tacrine, ENA-713 and TAK-147 for the inhibition of brain and plasma cholinesterase activities in rats
The ID_{50} values were calculated by means of regression analysis using the software package SAS ver. 6.12®. Numbers in parenthesis represent 95% confidence limits.

Drugs	ID_{50} (μmol/kg)		Ratio of ID_{50} (Plasma/Brain)
	Brain	Plasma	
Donepezil	6.3 (4.8–7.9)	89.0 (46.9–273.3)	14.1
Tacrine	40.5 (32.8–49.9)	> 170	> 4.2
ENA-713	7.2 (6.0–8.7)	9.7 (7.0–14.0)	1.3
TAK-147	26.8 (24.2–30.4)	51.2 (33.5–92.6)	1.9

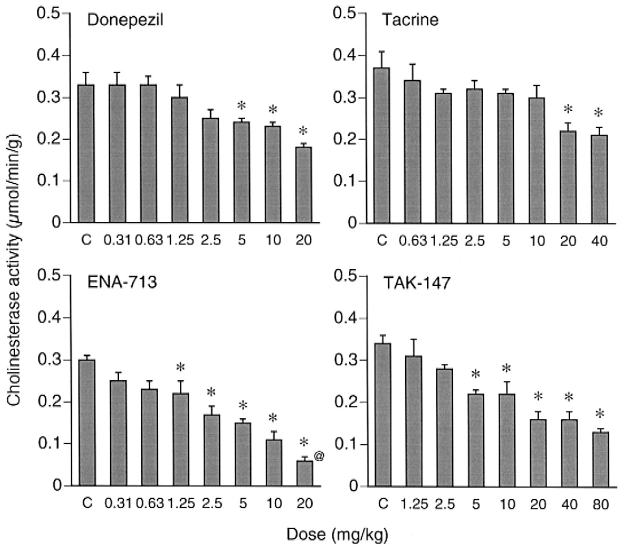

Fig. 2. Inhibitory effects of donepezil, tacrine, ENA-713 and TAK-147 on plasma cholinesterase activity in rats. Values represent the mean ± SE. $n = 5$ (@: $n = 4$). $^{*}P < 0.05$ vs. respective control (Dunnett's multiple comparison test).

temperature of 23 ± 1°C and relative humidity of 55 ± 10%, under a 12-h light/dark cycle (start at 0700 h) for at least 1 week before the experiment. The animals were given free access to food and water. All experiments were approved by the Animal Care and Use Committee of Eisai.

2.2. Drugs

Donepezil hydrochloride was supplied by Eisai Chemicals (Ibaraki, Japan) and tacrine by Sigma (St. Louis, MO, USA). ENA-713 and TAK-147 were synthesized by Eisai. [^{3}H]Acetylcholine iodide was purchased from New England Nuclear (Boston, MA, USA). All other chemicals were commercial products of reagent grade.

2.3. Measurement of cholinesterase activity

Donepezil, tacrine, ENA-713 and TAK-147 were each dissolved in distilled water before use. Control animals received distilled water. Drugs were administered orally in a volume of 5 ml/kg. One hour after the administration of the test compounds, animals were anesthetized by inhalation of a mixture of halothane (2%), nitrous oxide (70%) and oxygen (Balance), and blood was withdrawn from the ventral aorta. The whole brain, excluding the cerebellum and olfactory bulb, was excised and split into two hemispheres for measurement of cholinesterase activity and drug concentration. Cholinesterase activity in the brain and

Table 2
Concentrations of donepezil, tacrine, ENA-713 and TAK-147 in the rat brain and plasma after oral administration
Values represent the mean ± SE. $n = 5$.

Drug	Dose (mg/kg)	n	Brain (nmol/g)	Plasma (nmol/ml)	Ratio (Brain/Plasma)
Donepezil	0.31	5	0.032 ± 0.009	0.004 ± 0.001	8.4 ± 0.8
	0.63	5	0.102 ± 0.021	0.014 ± 0.003	7.7 ± 0.6
	1.25	5	0.180 ± 0.031	0.025 ± 0.004	7.1 ± 0.2
	2.5	5	0.511 ± 0.094	0.078 ± 0.012	6.6 ± 0.6
	5	5	0.782 ± 0.054	0.118 ± 0.010	6.7 ± 0.4
	10	5	1.036 ± 0.106	0.162 ± 0.010	6.4 ± 0.4
	20	5	1.429 ± 0.157	0.236 ± 0.024	6.1 ± 0.5
Tacrine	0.63	5	0.156 ± 0.024	0.002 ± 0.001	54.6 ± 2.7
	1.25	5	0.276 ± 0.036	0.006 ± 0.001	49.3 ± 1.6
	2.5	5	0.715 ± 0.122	0.019 ± 0.004	38.5 ± 2.8
	5	5	2.455 ± 0.370	0.089 ± 0.016	28.8 ± 2.5
	10	5	3.715 ± 0.327	0.133 ± 0.017	28.6 ± 1.7
	20	5	5.729 ± 0.394	0.406 ± 0.044	14.5 ± 1.2
	40	5	9.992 ± 2.576	0.599 ± 0.100	18.3 ± 5.7
ENA-713	0.31	5	0 ± 0	0 ± 0	–
	0.63	5	0 ± 0	0 ± 0	–
	1.25	5	0 ± 0	0 ± 0	–
	2.5	5	0 ± 0	0 ± 0	–
	5	5	0 ± 0	0 ± 0	–
	10	5	0.039 ± 0.019	0.006 ± 0.003	9.4 ± 3.3
	20	5	0.557 ± 0.285	0.082 ± 0.056	5.5 ± 0.4
TAK-147	1.25	5	0.074 ± 0.007	0.004 ± 0.000	20.6 ± 0.7
	2.5	5	0.154 ± 0.016	0.012 ± 0.002	12.8 ± 1.1
	5	5	0.199 ± 0.039	0.019 ± 0.003	10.6 ± 1.5
	10	5	0.642 ± 0.135	0.092 ± 0.018	7.0 ± 1.0
	20	5	1.512 ± 0.212	0.214 ± 0.018	7.3 ± 1.2
	40	5	2.557 ± 0.183	0.291 ± 0.045	9.6 ± 1.4
	80	5	3.806 ± 0.262	0.347 ± 0.037	11.3 ± 1.1

plasma was measured using the radiometric method of Thomsen et al. (1989), as modified by Sherman (1991). The brain hemispheres were homogenized, and plasma was diluted in four volumes of ice-cold assay buffer (2 mM HEPES buffer containing 0.5% Triton X-100, pH 7.4). Fifty microliters of brain homogenate or diluted plasma was added to a scintillation vial and incubated for a few minutes at 25°C. The reaction was initiated by the addition of 50 μl of [^{3}H]acetylcholine mixture [5 mM acetylcholine (using nonradiolabeled compound to make 3.7 kBq per assay), 0.1 M Tris–HCl (pH 7.4), 0.24 M NaCl]. After incubation for 1 min (for brain) or 10 min (for plasma) at 25°C, the reaction was terminated by the addition of 100 μl of stop solution (1 M chloroacetic acid, 2 M NaCl, 0.5 N NaOH). Four milliliters of scintillation cocktail [toluene containing 10% isoamyl alcohol, 0.51% 2,5-diphenyloxazole (PPO) and 0.03% 1,4-bis(5-phenyl-2-oxazolyl)-benzene (POPOP)] was added to each reaction tube and radioactivity was counted with a liquid scintillation counter.

2.4. Measurement of brain and plasma concentrations of donepezil, tacrine, ENA-713 and TAK-147

Brain and plasma concentrations of donepezil, tacrine, ENA-713 and TAK-147 were measured by LC-MS/MS. The brain hemisphere was homogenized in nine volumes of distilled water. Then, 0.1 ml of 0.001 M HCl and 0.1 ml of internal standard solution (1.1 nmol/ml of (*R*,*S*)-1-benzyl-4-[2-[(5,6-dimethoxy-1-indanon)-2-yl]-ethyl]piperidine hydrochloride) were added to 0.5 ml of each brain homogenate or plasma sample. Next, 5.0 ml of ethyl acetate was added, and the samples were shaken for 10 min. After centrifugation ($1800 \times g$, 5 min, 4°C), the organic phase was collected. Then, 5.0 ml of ethyl acetate was added again to the samples, and the above manipulations were repeated. The organic phases were combined and dried by blowing nitrogen at 40°C. The dried residue was dissolved in 0.2 ml of 0.1 M HCl, and this solution was injected into the LC-MS/MS system. ESI-MS/MS was carried out on a Finnigan MAT TSQ7000 (Finnigan MAT Instrument, Tokyo, Japan) mass spectrometer equipped with an LC system. The spectrometer was set to admit the protonated molecules $[M + H]^+$ at m/z 380 (donepezil), m/z 199 (tacrine), m/z 251 (ENA-713), m/z 377 (TAK-147) and m/z 394 (internal standard), with monitoring of the product ions at m/z 91 (donepezil), m/z 144 (tacrine), m/z 206 (ENA-713), m/z 91 (TAK-147) and m/z 91 (internal standard). The limits of quantification for the four cholinesterase inhibitors were set at 0.01 nmol/g or nmol/ml.

2.5. Statistical analysis

Data were analyzed using Dunnett's multiple comparison test or Student's *t*-test. A P value of less than 0.05 was considered significant. ID_{50} values were calculated by means of regression analysis. Statistical analysis was conducted using the software package SAS ver. 6.12® (SAS Institute Japan, Tokyo, Japan), available on the statistical analysis support system.

3. Results

3.1. Effects of donepezil, tacrine, ENA-713 and TAK-147 on brain and plasma cholinesterase activities

Oral administration of donepezil, tacrine, ENA-713 or TAK-147 caused dose-dependent inhibition of brain cholinesterase activity (Fig. 1). Minimum effective doses of these drugs were 0.63, 2.5, 1.25 and 5 mg/kg, respectively. Maximal degrees of inhibition produced at the highest dose of these drugs were 78, 67, 92 and 76%, respectively. One of the animals treated with ENA-713 at the highest dose died with severe cholinergic symptoms. The ID_{50} values of these compounds for brain cholinesterase activity were 6.3, 40.5, 7.2 and 26.8 μmol/kg, respectively (Table 1). Plasma cholinesterase activity was also inhibited by these cholinesterase inhibitors in a dose-dependent manner (Fig. 2). Minimum effective doses of these drugs were 5, 20, 1.25 and 5 mg/kg, respectively. Maximal degrees of inhibition produced at the highest dose of these drugs were 45, 43, 80

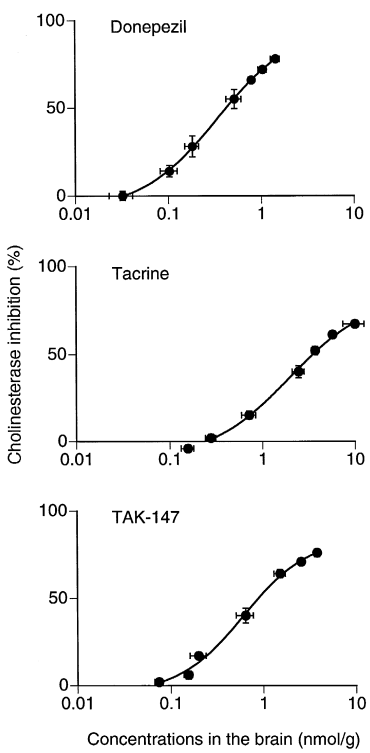

Fig. 3. Relation between cholinesterase inhibition and brain concentration of donepezil, tacrine or TAK-147 after oral administration. Cholinesterase inhibition is expressed as a percent inhibition, calculated based on the cholinesterase activity of the respective control as 100%. Values are mean ± SE.

and 62%, respectively. The ID_{50} values for plasma cholinesterase activity were 89.0, > 170, 9.7 and 51.2 μmol/kg, respectively (Table 1). Thus, the ratios of the ID_{50} (plasma/brain) were 14.1, > 4.2, 1.3 and 1.9, respectively (Table 1).

3.2. Brain and plasma concentrations of donepezil, tacrine, ENA-713 and TAK-147

Concentrations of donepezil, tacrine, ENA-713 and TAK-147 in the brain and plasma are shown in Table 2. Brain and plasma concentrations of donepezil, tacrine and TAK-147 after oral administration increased dose-dependently. The ratios of the concentrations (brain/plasma) of these compounds were 6.1–8.4 for donepezil, 14.5–54.6 for tacrine and 7.0–20.6 for TAK-147. In contrast, the brain and plasma concentrations of ENA-713 at all doses, except for the two highest doses, were below the quantification limit. A plot of brain concentration vs. enzyme inhibition for each drug is shown in Fig. 3. The values of 50% inhibitory concentration of the three drugs in the brain were 0.42, 3.5 and 1.1 nmol/g, respectively.

4. Discussion

In this study, donepezil inhibited rat brain cholinesterase activity more potently than tacrine or TAK-147, and was almost equipotent to ENA-713 under the same experimental conditions. We previously examined the effects of donepezil, tacrine and ENA-713 on extracellular acetylcholine concentration in the hippocampus of rats (Kosasa et al., 1999a). The increase (%) of the extracellular acetylcholine concentration about 1 h after oral administration of donepezil at 2.5 mg/kg was somewhat larger than that produced by tacrine at 5 mg/kg or ENA-713 at 2.5 mg/kg. Moreover, we have reported that the effect of donepezil on the extracellular concentration of acetylcholine in the cerebral cortex of rats was more potent than that of tacrine or TAK-147 (Kosasa et al., 1999b). The results of the present study are in good agreement with those findings. Thus, it is suggested that total cholinesterase activity in the brain reflects acetylcholinesterase activity in cholinergic nerves, and consequently, that the inhibitory effect of these drugs on cholinesterase activity in the brain is a valid index of the activation of the central cholinergic nervous system by the drugs.

Moreover, the 50% inhibitory concentration of the drugs for brain cholinesterase activity was compared with the in vitro IC_{50} values for acetylcholinesterase. The 50% inhibitory concentrations for brain cholinesterase activity of donepezil, tacrine and TAK-147 were 0.42, 3.5 and 1.1 nmol/g, respectively. The in vitro IC_{50} values of donepezil and tacrine were reported to be 5.7 and 80.6 nM, respectively (Rogers et al., 1991), while the IC_{50} values of tacrine and TAK-147 were given as 153 and 51.2 nM, respectively, in another study (Hirai et al., 1997). The potency ratios of these drugs for ex vivo cholinesterase inhibition obtained in this study were consistent with those for in vitro acetylcholinesterase inhibition (Table 3). These results indicate that the inhibitory effect on cholinesterase activity in the brain after oral administration of these drugs can be explained in terms of their in vitro activities for acetylcholinesterase inhibition and their concentrations in the brain.

On the other hand, the relative potency for the inhibition of plasma cholinesterase activity is ENA-713 > TAK-147 ≧ donepezil ≧ tacrine. The ratio of cholinesterase inhibition in the brain to that in plasma is likely to reflect the ratio of the clinical effect to potential side effects. This ratio was higher for donepezil and tacrine than for ENA-713 and TAK-147. Clinical adverse effects of cholinesterase inhibitors are mainly peripheral symptoms acutely produced by activation of the peripheral cholinergic nervous system, which is probably due to inhibition of acetylcholinesterase, not butyrylcholinesterase activity. This might suggest that inhibition of acetylcholinesterase activity is a more suitable parameter than inhibition of total plasma cholinesterase activity for prediction of the adverse effects of cholinesterase inhibitors. However, it has been suggested that cholinesterases play roles not related to the cholinergic nervous system (Chatonnet and Lockridge, 1989; Massoulié et al., 1993). For example, butyrylcholinesterase is considered to serve as a scavenging enzyme in the detoxification of natural compounds because it is involved in the degradation of drugs such as succinylcholine, heroin, cocaine and physostigmine (Massoulié et al.,1993). Moreover, it has been reported that inhibition of butyrylcholinesterase, under conditions where acetylcholinesterase is inhibited, results in potentiation of cholinergic responses in tracheal smooth muscle in vitro (Adler and Filbert, 1990). Thus, it is important to evaluate the overall influence of drugs on acetylcholinesterase and bu-

Table 3
Comparison between in vitro and ex vivo 50% inhibition concentrations for brain cholinesterase activity of donepezil, tacrine and TAK-147

Drug	In vitro[a]		In vitro[b]		Ex vivo	
	(nM)	(ratio)	(nM)	(ratio)	(nmol/g)	(ratio)
Donepezil	5.70 ± 0.20	0.07	–	–	0.42 (0.41–0.44)	0.12
Tacrine	80.60 ± 0.02	1	153 ± 4.0	1	3.5 (3.0–4.3)	1
TAK-147	–	–	51.2 ± 12	0.33	1.1 (0.66–2.9)	0.31

[a] IC_{50} values for acetylcholinesterase in the rat brain are taken from Rogers et al. (1991).

[b] IC_{50} values for acetylcholinesterase in the rat cerebral cortex are taken from Hirai et al. (1997). The values of ex vivo 50% inhibition concentration for brain cholinesterase activity were calculated by means of regression analysis using the software package SAS ver. 6.12®. Numbers in parenthesis represent 95% confidence limits.

tyrylcholinesterase in peripheral tissues from the standpoint of drug safety.

The preferential inhibition of brain cholinesterase activity is, in part, based on the ratio of drug concentrations in the brain vs. plasma. Another factor is the selectivity for acetylcholinesterase over butyrylcholinesterase, since cholinesterase in the rat brain is mostly acetylcholinesterase and cholinesterase in the rat plasma consists of both enzymes (Edwards and Brimijoin, 1982; Traina and Serpietri, 1984). In other words, a selective acetylcholinesterase inhibitor cannot completely inhibit rat plasma cholinesterase activity. The preferential inhibition of brain cholinesterase activity by donepezil is considered to be due to the additive effect of the high ratio of drug concentrations in the brain vs. plasma and the strong selectivity for acetylcholinesterase over butyrylcholinesterase (Rogers et al., 1991). Because tacrine is non-selective with respect to acetylcholinesterase and butyrylcholinesterase (Hunter et al., 1989; Rogers et al., 1991), its preferential inhibition of brain cholinesterase activity may be mainly explained by the ratio of drug concentrations in the brain vs. plasma, which is the highest among the drugs examined. The low selectivity of ENA-713 for brain cholinesterase is considered to be due to non-selective inhibition of acetylcholinesterase and butyrylcholinesterase, because the concentration ratio of ENA-713 in the brain vs. plasma is almost the same as that of donepezil at the two highest doses. The brain vs. plasma concentration ratio of TAK-147 was somewhat higher than that of donepezil. Moreover, TAK-147 exhibits selective inhibition of acetylcholinesterase over butyrylcholinesterase (Hirai et al., 1997), like donepezil. Thus, the low selectivity of TAK-147 for brain cholinesterase cannot be explained in terms of the observed brain and plasma concentrations of the drug and the selectivity for acetylcholinesterase. Previously, we observed that TAK-147 produced fasciculation at same dose as donepezil, although the effect of TAK-147 on the extracellular acetylcholine concentration in the cerebral cortex of rats was about four times less potent than that of donepezil (Kosasa et al., 1999b). The result of the present study is consistent with our previous findings. Since the inhibitory effect of TAK-147 on brain cholinesterase can be explained by its concentration in the brain and its in vitro inhibitory activity on acetylcholinesterase, it appears that the inhibitory effect of TAK-147 on plasma cholinesterase is more potent than would be expected from its plasma concentration. It was reported that TAK-147 is rapidly eliminated from the blood, in contrast to its prolonged residence in the brain after oral administration (Miyamoto et al., 1996). These findings suggest that active metabolite(s) of TAK-147 produced in the blood may contribute to the inhibitory effect of TAK-147 on cholinesterase activity in plasma after oral administration.

In spite of the clear inhibitory effect on brain cholinesterase, the brain concentration of ENA-713 was below the quantification limit at all doses except for the two highest doses. Enz et al. (1993) have also reported significant inhibitory effect of ENA-713 on cholinesterase activity in different brain regions after subcutaneous injection of the drug. ENA-713 is a carbamate-based cholinesterase inhibitor like physostigmine or neostigmine. This type of cholinesterase inhibitor associates with and carbamylates the active site of cholinesterase, thereby deactivating the enzyme, and is itself hydrolyzed. In contrast to the acetylated cholinesterase, which is formed during hydrolysis of acetylcholine, carbamylated cholinesterase is far more stable (Anand and Gharabawi, 1996). This may be one of the reasons why ENA-713 shows potent brain cholinesterase inhibition even though the brain concentration of the drug is very low.

In the current study, donepezil and tacrine produced more potent inhibition of cholinesterase in the rat brain than had been found in our previous study. We reported that donepezil, at oral doses of 3 and 10 mg/kg, and tacrine, at oral doses of 10 and 30 mg/kg, inhibited cholinesterase activity in the brain by 70% and 52%, and 76% and 77%, respectively (Rogers et al., 1991). The difference of the results between the two studies is considered to be due to the difference in the methods used for the measurement of cholinesterase activity. In the previous study, cholinesterase activity was measured using Ellman's method (Ellman et al., 1961). On the other hand, radiometric assay was employed in this study. It is known that the degree of cholinesterase inhibition by a reversible cholinesterase inhibitor is decreased by dilution of the assay medium (Hunter et al., 1989; Dawson, 1990). In Ellman's method, tissue homogenate was diluted with an excess of assay buffer, so the inhibitory effect of drugs would have been underestimated. The radiometric assay allowed us to reduce the volume of assay buffer. In the present study, in order to minimize the dilution effect, the homogenized brain and plasma were finally diluted in 10 volumes of assay buffer. However, it should be noted that inhibitory effects of the reversible cholinesterase inhibitors, donepezil, tacrine and TAK-147, may still be underestimated in the present study. ENA-713 produced more complete inhibition of cholinesterase activity in the brain and plasma than the other inhibitors, which may be explained by the pseudo-irreversible nature of its action (Anand and Gharabawi, 1996). The consequences of the dilution effect should be considered in interpreting the results of ex vivo cholinesterase inhibition experiments.

Behavioral studies have shown that donepezil, in the dose range of 0.5–2.0 mg/kg, significantly ameliorates performance deficits in several learning and memory tasks in rats, including eight-arm radial maze impairments after scopolamine and passive avoidance deficits produced by lesions of the nucleus basalis magnocellularis (Rogers et al., 1991). There have been discrepancies between the doses of donepezil reported to be effective in learning and memory studies and those required for inhibition of brain cholinesterase activity measured by Ellman's method (Ell-

man et al., 1961). In the present study, using radiometric assay, significant inhibition of cholinesterase activity in the brain was observed at a dose as low as 0.63 mg/kg, so that the apparent discrepancy is at least partly resolved. This result supports the idea that the ameliorating effect of donepezil on performance deficits in several learning and memory tasks in rats is based on inhibition of brain cholinesterase activity.

In summary, oral administration of donepezil dose-dependently inhibited brain and plasma cholinesterase activity in rats. The effect of donepezil on brain cholinesterase activity was more potent than that on plasma cholinesterase activity. Further, donepezil inhibited brain cholinesterase activity more potently than tacrine or TAK-147, and more preferentially in comparison with plasma cholinesterase activity than ENA-713 or TAK-147. The relative potencies of donepezil, tacrine and TAK-147 for inhibition of brain cholinesterase were well explained by the observed brain concentrations and the in vitro activities of the drugs. These results suggest that orally administered donepezil satisfactorily penetrates into the brain and inhibits cholinesterase there, and that donepezil is a potent and selective inhibitor of brain cholinesterase in comparison with plasma cholinesterase in vivo, and should be useful for the treatment of Alzheimer's disease.

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