CNS Drug Reviews
Vol. 7, No. 4, pp. 369–386

Eptastigmine: Ten Years of Pharmacology, Toxicology, Pharmacokinetic, and Clinical Studies

Daniela Braida and Mariaelvina Sala

Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan, Italy

Key Words: Acetylcholine—Alzheimer's disease—Cholinesterase inhibitors—Cognition—Eptastigmine.

ABSTRACT

Eptastigmine (heptyl-physostigmine tartrate) is a carbamate derivative of physostigmine in which the carbamoylmethyl group in position 5 of the side chain has been substituted with a carbamoylheptyl group. *In vitro* and *ex vivo* results suggest that eptastigmine has a long-lasting reversible brain cholinesterase (i.e., acetylcholinesterase and butyrylcholinesterase) inhibitory effect. When administered *in vivo* to rodents by various routes, eptastigmine inhibits cerebral acetylcholinesterases (AChE) and increases acetylcholine (Ach) brain levels by 2500–3000%, depending on the dose. This effect leads to an improvement in the cerebral blood flow in the ischemic brain, excitatory and inhibitory effects on the gastrointestinal tract and to a protection from acute soman and diisopropylfluorophosphate intoxication.

Eptastigmine, by either acute or chronic administration, has been found to have memory enhancing effects in different species of normal, aged and lesioned animals. It also restored to normal the age-related increase of EEG power without affecting spontaneous motor activity.

Clinical investigations on more than 1500 patients with Alzheimer's disease demonstrated that eptastigmine significantly improved cognitive performance (as assessed by the cognitive subscale of the Alzheimer's Disease Assessment Scale) as compared with placebo. This improvement was most evident in patients with more severe cognitive impairment at the baseline. The relationship between patient performance and average steady-state AChE inhibition was described by an inverted U-shaped dose-response curve.

Address correspondence and reprint requests to Dr. Mariaelvina Sala at the Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan, Via Vanvitelli 32, 20219, Milan, Italy.
Tel: +39-2-58357042; Fax: +39-2-58357036; E-mail: msala@mailserver.unimi.it

Pharmacokinetic studies have revealed that after oral administration eptastigmine is rapidly distributed to the tissues and readily enters the CNS, where it can be expected to inhibit AChE for a prolonged period.

Eptastigmine is generally well tolerated and the majority of adverse events (cholinergic) were mild to moderate in intensity. However, the adverse hematologic (granulocytopenia) effects reported in two studies have resulted in the suspension of further clinical trials.

INTRODUCTION

Eptastigmine [1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrole(2,3-b)indol-5-ol heptylcarbamate tartrate] was originally synthesized in 1985 (11) in an attempt to replace two older acetylcholinesterase inhibitors (AChEIs): physostigmine and tacrine. This approach represented the first pharmacological attempt to increase cholinergic function in patients with Alzheimer's disease (AD). Several authors (3,39) reported discordant results using these drugs and raised concerns over their tolerability. The unfavorable kinetic characteristics of physostigmine include short half-life, rapid metabolism and variable plasma concentration after oral administration. Both compounds are poorly tolerated. Physostigmine has a narrow cholinomimetic therapeutic window, while tacrine may produce liver injury. In this context, the search for an AChEI with a similar potency, but better pharmacokinetic profile (longer half-life, higher bioavailability and brain levels) and lower toxicity than that of the existing drugs, led to the development of eptastigmine (5,45).

CHEMISTRY

Eptastigmine is a derivative of physostigmine in which the carbamoylmethyl group in position 5 of the chain has been substituted with a carbamoylheptyl group (Fig. 1). It is an odorless, bitter tasting, white to off-white, crystalline powder with a melting point of 121 to 125°C. It is very soluble in water, methanol, and chloroform. With the exception of rapid degradation in the presence of 1 N NaOH, the substance withstood hours to days of exposure to the test conditions with only limited signs of degradation (65).

PHARMACODYNAMIC PROPERTIES

In Vitro and *Ex Vivo* Studies

Eptastigmine is a competitive AChEI with a $K_i = (1 \pm 0.5) \times 10^{-7}$ M. The inhibition is instantaneous and does not diminish with the prolonged incubation of the drug with the enzyme (13).

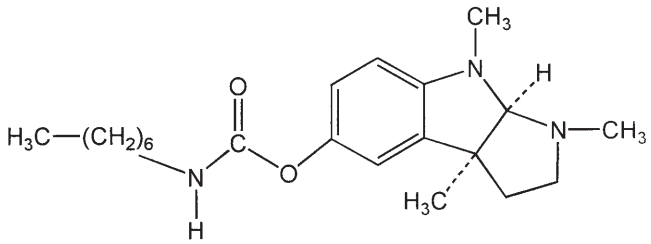

Fig. 1. Chemical structure of eptastigmine.

The acetylcholinesterase (AChE) inhibitory activity of eptastigmine was studied in an *in vitro* assay system using particulate membrane fractions. This system permits comparison of inhibition and recovery kinetics of human red blood cells (RBC; primarily globular dimer) and brain (primarily globular tetramer) membrane-bound forms of AChE. The IC_{50} of eptastigmine after 1-h incubation was similar for the two forms ($IC_{50} = 7.6 \times 10^{-9}$ M for the brain AChE and 1.2×10^{-8} M for RBC AChE). However, the recovery rate of RBC and brain membrane-bound AChE were different. AchE activity in RBC membranes recovered with a half-life of 7.39 h while no recovery was observed for brain enzyme after 24 h incubation. Following inhibition with physostigmine brain enzyme activity recovered within 24 h. The longer duration of action of eptastigmine might have been due to the attachment of a six-carbon carbamyl chain of eptastigmine to a hydrophobic portion that is adjacent to the active site of the AChE (48).

In *ex vivo* experiments in rhesus monkeys up to 86% of AChE inhibition was achieved in RBC after treatment with eptastigmine at a behaviorally active dose of 8 mg/kg p.o. (55).

Eptastigmine (0.5 to 4 mg/kg i.p.) caused dose-dependent reversible inhibition of rat brain AChE activity. It was less potent than physostigmine. Fifty percent inhibition of the enzyme activity was obtained with 1.39 mg/kg of eptastigmine versus 0.19 mg/kg of physostigmine (12). However, at equally effective doses eptastigmine had a longer duration of action than physostigmine. At 5 mg/kg i.m. eptastigmine inhibits AChE in whole rat brain, in various brain regions as well as in RBC. It also inhibits butyrylcholinesterase in plasma. The maximal inhibition of AChE is reached in whole brain (82%) and in RBC (75%) at 60 min, while the maximal inhibition of butyrylcholinesterase in plasma (58%) is reached at 120 min (25). In rats with ibotenic acid — induced lesions in the nucleus basalis magnocellularis eptastigmine (0.078 to 10 mg/kg s.c.) inhibited AChE activity dose-dependently. At two h after treatment the inhibition ranged from 20 to 60% (67).

The mammalian brain AChE exists in multiple molecular forms. The globular tetrameric G4 form and the monomeric G1 form are predominant in the human brain. Both the G1 and the G4 forms are important for neuronal function and ACh regulation. The G4 form is selectively decreased in AD. When pre-incubated in homogenized brain tissue of AD patients, eptastigmine preferentially inhibited the molecular G1 form of AChE, while physostigmine inhibited both forms with similar potency. This suggests a possible therapeutic application of a G1 selective inhibitor in AD since this form is relatively unchanged in AD brain (51,52).

The ability of eptastigmine to displace several different ligands from their binding sites was assessed in radioligand binding assays using rat brain homogenates. The compound

displayed an intermediate effect between tacrine and physostigmine at both muscarinic and nicotinic binding sites. The displacing effect was stronger at [^{3}H]QNB than at (–)[^{3}H]nicotine binding sites; the IC_{50} (μM) was 1.9×10^{-5} for muscarinic and 1.0×10^{-4} for nicotinic receptors (25). This finding suggests that the therapeutic effect of eptastigmine is mediated mainly through an activation of muscarinic receptors. In whole rat brain membrane fractions eptastigmine does not distinguish between different subtypes of neuronal nicotinic ACh receptors (1).

Eptastigmine (5 mg/kg) elevated acetylcholine (ACh) levels in some rat brain areas more than in others. The maximal increase in ACh levels (75%) in parietal cortex was reached 60 min after treatment. In frontal cortex and striatum the maximal increase in ACh levels 120 min after treatment reached 120 and 60%, respectively. In hippocampus and medula oblongata only transient and small (10–20%) increases in ACh levels were observed 5 min after administration of eptastigmine.

In superfused rat brain cortical slices, eptastigmine (0.1 μmol/L) increased amyloid precursor protein levels by 141% from the baseline. This finding suggests a neuroprotective effect of eptastigmine due to activation of processing of normal amyloidogenic soluble derivatives (APP). At this concentration eptastigmine inhibited AChE activity by 61% and caused a 40-fold increase in ACh levels in rat frontal cortex (46).

In Vivo Studies in Animals

The *in vivo* effects of eptastigmine on cerebral cholinesterase activity and brain ACh levels in rodents were studied by microdialysis. Eptastigimine, by various routes of administration, inhibited acetylcholinesterase and increased brain ACh levels (Table 1).

Eptastigmine at 2 and 5 mg/kg s.c. dose-dependently inhibited extracellular cholinesterase activity (70–80%) in rat cerebral cortex. The endogenous ACh levels were increased by eptastigmine 2500–3000% at 60 or 90 min in a dose-dependent manner (20,21). The increase in brain ACh was followed by a slight elevation of extracellular norepinephrine (NE) (24–29%) and dopamine (74% at the lowest dose). In comparison, physostigmine (30–300 mg/kg) produced its maximal increase in ACh levels between 30 and 60 min after treatment. The increase in ACh levels was accompanied by a significant increase in the levels of biogenic amines. Six hours after treatment ACh levels remained increased above baseline with eptastigmine, but not physostigmine. The increase in ACh levels (943 and 4024%) produced by physostigmine was accompanied by cholinesterase inhibition of about 50% suggesting that ACh levels are not directly related to the inhibition of AChE. Consequently, AChE inhibition cannot accurately predict the effect of eptastigmine on the levels of extracellular ACh in cerebral cortex. In addition, the co-administration of eptastigmine (2 mg/kg s.c.), but not physostigmine, with idazoxan (given centrally or peripherally) produced a more sustained effect on ACh cortical levels than did eptastigmine alone. This suggests that the blockade of α_2-heteroceptors by idazoxan may prevent the negative modulation of NE on ACh release (20).

At 5 mg/kg p.o. eptastigmine produced maximal inhibition of AChE (50%). One hour after treatment, ACh levels were increased by about 2000%, NE levels by 151% and dopamine levels by 195% (70). There was a continued steady rise in the levels of ACh which reached C_{max} at 5 to 6 h after treatment.

Finally, eptastigmine, 50 μM intracortically, produced a 6700% increase in ACh brain levels and a 52% increase in dopamine levels. Physostigmine had no effect on dopamine

levels even when it increased ACh levels by 9700% (21). Since cholinergic agonists increase dopamine turnover and release *in vivo*, this eptastigmine-induced reciprocal interaction between dopamine and ACh suggests an additive therapeutic effect.

Transdermal administration of eptastigmine has also been examined in the rat (47). Brain AChE inhibition of 23 to 34% was obtained 24 h after administration of 24 μmol of the drug in either a hydrophobic or hydrophilic transdermal drug delivery system. Enzyme inhibition was similar 3 to 5 days after treatment.

The effect of subchronic administration of eptastigmine (0.6 mg/kg s.c. daily for 15 days) on cortical extracellular ACh levels in aged rats was also investigated. Following a challenge dose of eptastigmine (administered 24 h after the last treatment), the release of ACh was faster and more pronounced in treated versus controls rats (604 vs. 457 fmol/30 min). In the same animals AChE activity in the striatum decreased significantly (20%) (27). These results suggest that moderate inhibition of AChE in the brain is able to maintain high levels of extracellular ACh in the cortex of aged rats. This finding contradicts the currently accepted opinion that, given the large amount of AChE present in extracellular space, 70 to 80% inhibition of the enzyme is necessary to appreciably increase synaptic ACh levels.

In rhesus monkeys eptastigmine (8 mg/kg p.o.) produced a long-lasting hypothermia of up –1.6°C at 60 to 300 min after treatment. This effect was accompanied by 86% inhibition of AChE in RBC (55).

In rats at 2 mg/kg s.c. eptastigmine, corticosterone levels were increased after acute but not after short-term ad-

TABLE 1. Effects of eptastigmine or physostigmine on extracellular levels of ACh, biogenic amines and cholinesterase inhibition in the rat brain

Drug	Dose (mg/kg)	Route	Peak (min)	ACh_{max} (% increase)	NE_{max} (% increase)	DA_{max} (% increase)	5-HT_{max} (% increase)	Maximal % AChE inhibition	Side effects
Eptastigmine[a]	2	s.c.	60	2497	24	74	n.s.	74	+++
Eptastigmine[a]	5	s.c.	90	2964	29	n.s.	n.s.	88	+++
Physostigmine[a]	0.03	s.c.	30	943	20	70	n.s.	42	++++
Physostigmine[a]	0.3	s.c.	30	4024	74	117	n.s.	57	++++
Eptastigmine[b]	5	p.o.	60	2000	151	195	n.s.	50	++
Eptastigmine[b]	5	p.o.	360	2100	60	n.s.	n.s.	–	–
Eptastigmine[a]	50 µM	i.c.	180	6709	n.s.	52	n.s.	50	++
Physostigmine[a]	50 µM	i.c.	90	9720	n.s.	n.s.	n.s.	–	++

i.c. = intracortical; n.s. = not significant; ++, slight fasciculations and tremor; +++, fasciculations, tremor and splay; ++++, salivation, chewing, fasciculations, tremor and splay.

Adapted from refs. [a] 21 and [b] 7.

ministration (6 days). This finding suggests increased corticosterone levels is a short-lasting effect that may be insignificant during long-term therapy (17).

Eptastigmine (0.5, 1, and 2 mg/kg i.m.) increased basal and GHRH-stimulated GH release in young and old dogs. The good activity shown in old dogs suggests a potential use of this drug to reverse the age-dependent decline in GH secretion (19).

Eptastigmine at 0.5, 4, 8, 12, and 20 mg/kg p.o. had an excitatory and an inhibitory effect on rat gastrointestinal transit. There was, however, an inverted U-shaped dose-response curve. Similar results were obtained with tacrine and donepezil (10). The biphasic effect of eptastigmine was peripherally mediated through both muscarinic and nicotinic receptors. However, the stimulatory and inhibitory doses were higher than those required to improve memory in the same animal species.

The ability of eptastigmine to improve cerebral blood flow in the ischemic brain was tested in rats following tandem occlusion of the left middle cerebral and the common carotid arteries. With the exception of ischemic core, eptastigmine (3 mg/kg i.v.) improved cerebral blood flow in most regions of the cortex. The effect was more pronounced at 24 h than at 2 h after ischemia (57). Cerebrovascular and metabolic effects were also tested in normal rats. A single intravenous injection of 0.5 to 3.0 mg/kg of eptastigmine produced a dose-dependent increase in regional cerebral blood flow (56). This increase was correlated with the cerebral glucose utilization. The slope of glucose utilization was steeper in eptastigmine treated as compared to control rats with the utilization rate peaking 40 min after treatment. At 3 mg/kg i.m. eptastigmine increased cerebral cortical blood flow and strongly inhibited AChE (41). These studies demonstrated an important role of endogenous ACh in the control of cerebral perfusion.

At 0.98 mg/kg i.v. eptastigmine, given 10 min prior to different organophosphate compounds, protected better than physostigmine against acute soman and diisopropylfluorophosphate (DFP) but not sarin intoxication (62–64). The combination of eptastigmine and an organophosphate hydrolase (phosphotriesterase) was found to represent a novel therapy against DFP intoxication in mice. Presumably, eptastigmine, by preventing the binding of DFP to cholinesterases, caused an elevation of free DFP levels in body fluids. This promoted phosphotriesterase hydrolysis of DFP (61). This finding suggests that eptastigmine, unlike tacrine, may have prophylactic efficacy against organophosphate intoxication.

EFFECTS ON MEMORY, LEARNING, AND BEHAVIOR

The enhancing effect of eptastigmine on memory was investigated in different species of young, aged and lesioned animals using working and reference memory tasks. Eptastigmine at 0.625 to 5 mg/kg i.p. significantly enhanced passive avoidance behavior in mice. At the highest dose step-through latency, assessed at 24 h after training, increased by 72% (12). Similar effects were obtained in rats (14). When given at 1 to 3 mg/kg s.c. to mice or at 8 mg/kg i.p. to rats, eptastigmine completely reversed scopolamine-induced deficit in a range of behavioral tests. These tests of long-term and working memory included conditioning suppression of drinking procedure and random interval schedule re-

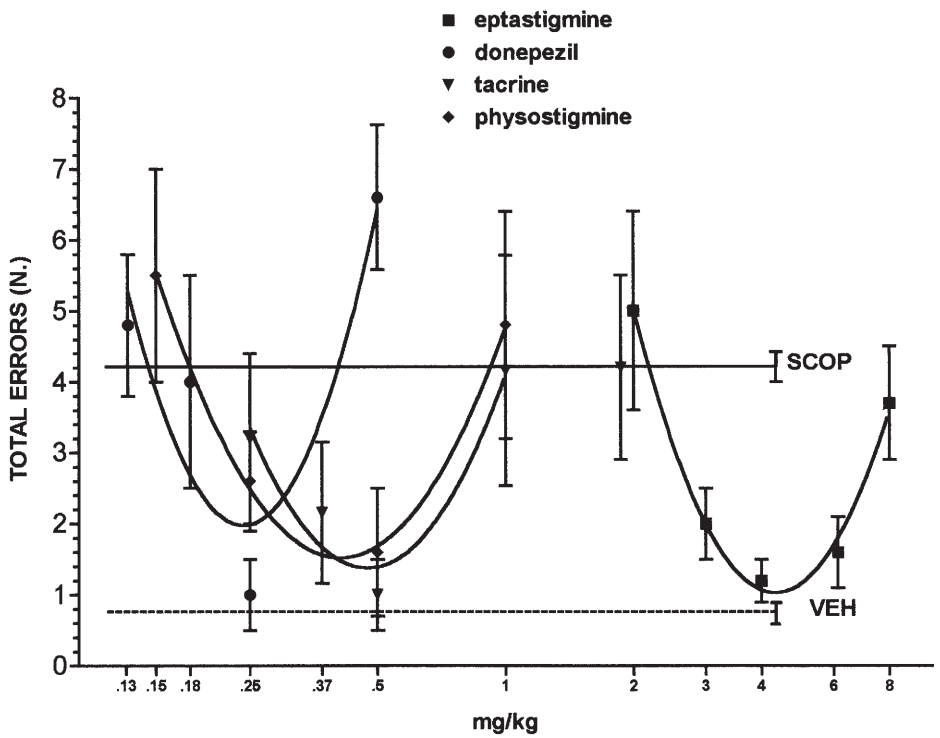

Fig. 2. Parabolic regression lines of the total number of errors (mean ± S.E.M.) plotted against the log of orally administered doses of different AChEIs. Scopolamine, 0.25 mg/kg s.c. (SCOP) (mean ± S.E.M.); vehicle (VEH) represents the pooled mean ± S.E.M. value for rats treated with saline s.c. and distilled water, p.o. on the day before pharmacological treatment.

sponse rates (23,24). In rats, at 2 mg/kg i.p., eptastigmine partially reduced scopolamine-induced deficit in a 14-unit T-maze training but at 1 or 3 mg/kg failed to produce significant effects (32).

At 2 to 8 mg/kg p.o., eptastigmine reversed scopolamine-induced working memory deficit in rats in 8-arm radial maze, but its dose-response curve had an inverted U-shape (Fig. 2). This effect was also observed with tacrine, donepezil or physostigmine. However, eptastigmine appeared to be more efficacious in selectively antagonizing cognitive deficit over a relatively wide range of doses when compared with the other AChEIs (8). Eptastigmine was also active in relieving memory impairment induced by a selective CB1 cannabinoid agonist, CP 55,940, in the same test (9).

Chronic treatment with eptastigmine (0.25 mg/kg p.o., b.i.d. for 30 days) improved the radial maze performance of 18 months old rats. This effect was maintained without any further treatment until 23 months of age (6). The performance in same age rats was also improved after a single dose (0.5 mg/kg p.o.) when a 2-h delay was allowed between the fourth and fifth choice. These findings suggest that in aged rats AChE inhibition has a positive effect on cognitive processes related to spatial memory through a selective cholinergic mechanism. An improved cognitive effect was also observed in an object recog-

TABLE 2. Effect of eptastigmine on different memory tasks in rodents and rhesus monkeys

Species	Test	mg/kg, route	Effect	Reference
Mice	Passive avoidance	1–5 i.p.	Performance as controls	12
	Passive avoidance	1–3 s.c.	Complete reversal of scopolamine-induced deficit	24
Rats	Passive avoidance	0.625–5 i.p.	Increased retention	14
	Passive avoidance	8 s.c.	Complete reversal of scopolamine-induced deficit	23
Rats	Suppression of drinking test	1–4 s.c.	Partial reversal of scopolamine-induced deficit	23
	Suppression of drinking test	8 s.c.	Complete reversal of scopolamine-induced deficit	24
Rats	Random Interval Training	4 s.c.	Block of lever pressing	23
Rats	Delayed matching–to-position	4 s.c.	Partial reverse of scopolamine-induced deficit	23
Rats	8-arm radial maze	2–8 p.o.	Inverted U-curve of scopolamine-induced deficit	8
	8-arm radial maze	6 p.o.	Complete reversal of cannabinoid-induced impairment	9
	14 unit T-maze	2 p.o.	Complete reversal of scopolamine-induced deficit	32
Lesioned rats	Morris water maze	0.2–1.25 s.c.	Failure to antagonize memory impairment	67
Old rats	8-arm radial maze	0.5 p.o. twice a day for 30 days	Complete reversal of delay-induced memory impairment	6
Old rats	Object recognition	0.6 s.c. for 15 days	Complete reversal of age-related spatial memory deficit	27
Rhesus	Spatial delay response task	0.2–0.9 i.m.	Complete reversal of scopolamine-induced deficit	55
monkeys		8 p.o.		54

nition test after subchronic administration of eptastigmine (0.6 mg/kg s.c. daily for 15 days) in 26 month old rats (27).

A trend towards improvement in the acquisition of Morris water maze task after eptastigmine (1.25 mg/kg, s.c.) was observed in rats with ibotenic acid–induced lesions in the nucleus basalis magnocellularis (67). In rhesus monkeys eptastigmine (0.1 to 0.9 mg/kg i.m. or 6 to 8 mg/kg p.o.) fully reversed scopolamine-induced cognitive impairment in a spatial delayed response task. This effect differed from that of physostigmine and from that of some muscarinic agonists which only partially affected it (54,55).

Eptastigmine affected different components of behavior and EEG in rodents. EEG cortical activity was recorded in mice after a single dose of eptastigmine, 10 mg/kg i.p. Its effect was characterized by an increased amplitude and a reduced frequency. This was interpreted as a sign of cholinergic activation (12). In DBA/2 mice eptastigmine (2 mg/kg i.p.) induced a cortically derived power increase in the 4.25- to 7-Hz frequency band, while at 6 mg/kg it produced a power decrease in the 7.25- to 12-Hz frequency band. Similar changes were observed in mice submitted to flash visual evoked potentials. Amplitudes of early and late components were enhanced, while amplitudes of middle components were depressed at all doses used (60). These findings might indicate that eptastigmine, in a very narrow dose range, is effective at modifying EEG and flash visual evoked potentials in terms of amplitude parameters connected with brain cholinergic function. In 27 to 30 month old rats the age-related increase of EEG mean cortical spectral power in slow wave delta and decrease in fast wave alpha and beta activities were restored to normal by eptastigmine. This effect occured in a dose-dependent manner at 0.5, 1, 2, and 4 mg/kg p.o. (7). These findings suggest a possible strategy to alleviate the severe slowing of neocortical EEG which accompanies the cognitive decline.

Spontaneous motor activity in mice was not modified by eptastigmine, at 0.1 to 3 mg/kg i.p. However, eptastigmine at 3 mg/kg antagonized scopolamine-induced stimulation of locomotor activity (12). This finding indicates that increased cholinergic function antagonizes the behavioral stimulation related to the anticholinergic effect of scopolamine. Similar results were obtained in rats, in which eptastigmine (8 mg/kg p.o.) reversed scopolamine-induced hypermotility (8). It is of interest to note, that at a memory enhancing dose (4 mg/kg), eptastigmine was ineffective in reducing scopolamine-induced hypermotility suggesting a selective action on memory function. The increased exploratory behavior observed in lesioned rats was reduced by eptastigmine (1.25 mg/kg) in an open field test (67). At 0.32 to 5 mg/kg i.p., eptastigmine reversed scopolamine's effect on olfactory exploration, but not on aggression. This would suggest a role for ACh in the development of social recognition as an olfactory memory (68).

Eptastigmine (0.625 to 10 mg/kg s.c.) produced dose-dependent decreases in the overall rate of reinforcement under a chained schedule of differential-reinforcement-of-high/low-rate reward in rats (42). At 2.89 mg/kg eptastigmine produced no behavioral deficits, but moderately inhibited AChE.

At 8 mg/kg i.p. eptastigmine produced a pronounced antinociceptive response in mice with a maximal effect 2 h after treatment, followed by a slow decline over the next 3 h (23). This finding suggests that brain cholinesterase inhibitory action of eptastigmine is slow in onset, but prolonged in duration.

ANIMAL TOXICITY

In acute oral and parenteral toxicity studies in mice, rats, and monkeys, the principal signs of toxicity were tremors, salivation, fasciculations, lacrimation, and diarrhea (Table 3). These signs are consistent with the excessive cholinergic activity induced by cholinesterase inhibition. The LD_{50} for eptastigmine i.p. was 35 mg/kg in mice as compared to 0.6 mg/kg i.p. for physostigmine (12) suggesting that this second generation cholinesterase inhibitor is less toxic than the first generation inhibitor, physostigmine.

PHARMACOKINETICS

Pharmacokinetics and tissue distribution of eptastigmine were studied in rats. The drug was administered at a single doses of 2 mg/kg i.v., 4 mg/kg i.m., and 4 or 8 mg/kg p.o. (58). Eptastigmine remained a long time in plasma (the terminal half-life was about 12 h) and was widely distributed in tissues (the volume of distribution was about 6L). Its brain levels were 4–22 times higher than plasma levels.

Pharmacokinetic studies in young and elderly volunteers as well as in AD patients, receiving 30 to 60 mg/day of eptastigmine for 4 weeks, indicated that the maximal plasma concentrations of the drug rarely exceed 1 ng/mL (15). In pharmacokinetic studies three male volunteers (22 to 34 years old) and six elderly subjects (63 to 84 years old) received eptastigmine at single doses of 30 to 40 mg p.o. Mean maximal plasma concentrations of the drug (C_{max} = 0.78 and 0.86 μg/L, respectively) peaked 1 to 1.4 h after treatment (2,66).

Eight male volunteers (23 to 28 years old) received single oral doses of eptastigmine, 10, 20, or 30 mg p.o. The plasma levels of the drug increased in a dose-related manner and area under the curve (AUC) values were 0.74, 3.61, and 6.25 μg/L per hour, respectively (69,34). In elderly volunteers, a 30 mg oral dose of eptastigmine was rapidly absorbed and distributed in tissues (distribution half-life 0.44 h) and then slowly eliminated (elimination half-life 12.1 h) (2). These values are probably underestimated since they were calculated on limited blood sampling (24 to 48 h). Assuming a terminal elimination half-life of 12 h

TABLE 3. Toxicity of acute eptastigmine in rodents and primates

Species	mg/kg, route	Cholinergic side effects	References
Rats	1.25 s.c.	Oral movements and muscle fasciculations	42
Rats	2 and 5 s.c.	Fasciculations, tremors and splay	21
Rats	5–10 s.c.	Moderate to marked salivation, body tremor, fasciculations, splay, chromodacryorrhea.	42,67
Rats	5 p.o.	Slight fasciculations and tremors	70
Rats	50 μM i.c.[a]	Slight fasciculations and tremors	21
Rhesus monkeys	6–8 p.o.	Emetic episodes	55

[a] Intracortically.

for unchanged eptastigmine in humans, a three times daily regimen may produce a three-fold accumulation of the drug in plasma. In cerebral cortex and in bone marrow the extent of accumulation could be considerably higher. The ingestion of food significantly reduced the bioavailability of eptastigmine (30 mg/kg). Food decreased the areas under the curve of RBC AChE inhibition from 0 to 8 h by 37% and the mean maximum inhibition of RBC AChE by 17% (4).

HUMAN PHARMACODYNAMIC STUDIES

Studies in healthy young volunteers showed that oral eptastigmine (10, 20, and 30 mg) dose-dependently inhibited RBC AChE activity (34). Peak enzyme inhibition (15, 30, and 36% with respective doses) occurred after 3, 2.9, and 3.6 h. Inhibition of plasma butyryl-cholinesterase activity was weak and did not appear to be dose related.

In elderly volunteers peak cholinesterase inhibition in plasma (17%) was reached within 2.7 h after administration of a 30 mg oral dose of eptastigmine. Peak enzyme inhibition in RBC (29%) was reached after 3.8 h (2). Significant inhibition of AChE was still observed in plasma (9%) and in RBC (14%) after 12 h. The estimated half-time for cholinesterase recovery was 12.4 h in plasma and 13.6 h in RBC.

Pharmacodynamic studies were also conducted in AD patients after repeated administration of eptastigmine. Since multiple blood samplings in AD patients may be problematic, an automated device has been developed that measures AChE from 10 μL capillary blood (18,49). The activity of the enzyme can be indirectly measured from the changes in pH generated by the hydrolysis of ACh. This non-invasive method permitted a precise estimate of pharmacodynamic parameters.

Eighty-one patients with AD received eptastigmine at 40 to 60 mg/day for 4 weeks, peak cholinesterase inhibition occurred 2 to 4 h after each treatment. At steady state, peak (54.5%) cholinesterase inhibition was reached after 4 days (16). A dose-dependent reduction of RBC cholinesterase activity was observed in 20 patients with AD after treatment with eptastigmine, 12, 20, or 28 mg p.o., three times daily, for 14 days (59). Basal forebrain cerebral blood flow increased by 5.5 to 17.1% in 4 of 8 patients with AD who received eptastigmine, 15 mg, p.o., 3 times daily for 4 weeks (53).

THERAPEUTIC TRIALS

Clinical results obtained over the past ten years with eptastigmine involved an overall exposure of more than 1500 patients. An earlier study examined the effects of two single doses of eptastigmine (20 and 32 mg) on scopolamine-induced cognitive deficits in 24 healthy male volunteers. At either dose eptastigmine did not significantly diminish or reverse scopolamine-induced impairments, even when a 23 to 37% of RBC AChE inhibition was obtained (40).

The results of six eptastigmine trials are summarized in Table 4. All studies examined cognitive, functional and global effects of eptastigmine in patients with mild to moderate

dementia (Mini-Mental State Examination (MMSE) = 10–26 score). A relationship between RBC AChE inhibition and the corresponding cognitive and behavioral effects in the individual AD patients was found. These studies indicate that at 20 mg, p.o., t.i.d. eptastigmine produces moderate steady-state AChE-inhibition (between 20 and 40%) associated with maximal cognitive and clinical improvement. These results are in line with those reported for other AChEIs (physostigmine, metrifonate and galanthamine) in which an inverted U-shape relationship between AChE inhibition and cognitive effect has been reported. These studies found no effect at low doses, improvement at moderate doses, and impairment at higher doses (28).

The clinical efficacy trials with eptastigmine in AD patients revealed an improvement in some tests on cognition, global function and activities of daily living. The cognitive improvement was obtained either after short- (1 to 6 months) or long-term treatment (2 years). In patients treated with eptastigmine the magnitude of cognitive effects measured with the Alzheimer's Disease Assessment Scale-Cognitive Subscale score (ADAS-cog) varied between 1.63 to 2.3 points as compared with placebo. The cognitive effect measured with the ADAS-cog scale for eptastigmine is similar to that obtained with other AChEI (tacrine, donepezil, rivastigmine, metrifonate, and galanthamine) (29). The ceiling value of approximately 5 ADAS-cog points average for patients with mild to moderate stages of the disease, was never reached with any of the AChEIs. It should be noted, that the positive cognitive effect of eptastigmine appeared to be greater in moderately and moderately to severely impaired patients. Similarly, tacrine was more effective in patients with low MMSE scores (10–17) than in those with high MMSE scores (18–26) (26).

The overall incidence of adverse events due to cholinergic stimulation that led to interruption of treatment was about 11–15%. Analysis of 6 months of data for different AChEIs (30,44) indicated that the number of dropouts in the eptastigmine group was comparable to that reported for donepezil- and metrifonate-treated patients. A higher percentage and most severe side effects (hepatotoxicity and general cholinergic toxicity) were seen with tacrine (55% of dropouts) and physostigmine (57% of dropouts). Taken together, these findings suggest that, although there are no difference in the benefits with different AChEIs, their adverse effects are likely to differ considerably.

HUMAN TOLERABILITY AND SAFETY

A maximal tolerated single dose of eptastigmine (32 mg/day) in elderly healthy volunteers has been reported to produce a mean RBC AChE inhibition of 45% (43). In five patients with AD eptastigmine was well tolerated up to a maximal dose of 48 mg three times daily (22,59). Some of the adverse effects (ataxia, dizziness, and fatigue) at the maximal tolerated dosage involved the CNS. In 25 of 74 patients taking eptastigmine (40 or 60 mg for 4 weeks) most side effects were cholinergic. They included transient mild nausea, vomiting, bradycardia, and ventricular extrasystoles. These side effects occurred when peak AChE inhibition was more than 50% after the first dose or 70% at the steady state. All clinical adverse events were transient and rated as mild or moderate. No laboratory or ECG abnormalities were noted (16). Similar results were obtained in a randomized, double-blind, placebo controlled study involving 320 AD patients treated for 25 weeks

TABLE 4. Clinical trials with eptastigmine in AD patients

Design	Dosage (mg oral)	No. Patients	Duration	Completers	Adverse effects in at least 2% of patients	Outcome	Results/Comments
Double-blind, randomized, placebo-controlled, unbalanced parallel-group. To assess safety, tolerability and preliminary efficacy in patients with probable AD (16)	20 b.i.d. or t.i.d. based on weight	103 (20 on placebo)	4 weeks 8 weeks (open extension phase)	94 (20 on placebo) 34 (incl. placebo)	Nausea, vomiting, fainting, bradycardia, reversible increase in liver transaminase, neutropenia associated with peak AChE inhibition exceeding 50%.	**IADL**: significant improvement; **CGIC**: significant improvement. **LMT, SWFT, TMT** significant improvement only in patients with RBC AChE inhibition between 25 and 40%.	Maximal cognitive improvement in patients with RBC AChE inhibition between 25 and 40% with an inverted U shape dose-response curve.
To assess heart rate variability and efficacy in AD patients (31)	10 t.i.d. orally	20 (10 on placebo)	6 months	20	Total spectral power significantly increased in patients with cognitive improvement.	**ADAS-Cog**: significant improvement	Total power increase was evident in all 5 responders, while 4 out of 5 non-responders showed a reduction of this value. Spectrum total power is a useful tool to discriminate the potential responders to cholinesterase inhibitor therapy.
Double-blind, placebo-controlled, unbalanced parallel-group. To assess safety and efficacy in probable AD patients (35)	10 and 15 t.i.d. orally	320 (106 on placebo)	25 weeks	315	Not different from placebo.	**ADAS-Cog**: 2.0 vs. placebo with 15 mg; **SBI**: 2.1 vs. placebo with 10 mg; **ADL**: 0.9 vs. placebo with 10 mg; **BP**: 0.6 vs. placebo with 10 mg	CIBIC-Plus reached statistical significance only in the most severely impaired patients (GDS = 4/5 score). Significant cognitive effect with 15 mg associated to AChE inhibition of 30%.

TABLE 4. Clinical trials with eptastigmine in AD patients

Design	Dosage (mg oral)	No. Patients	Duration	Completers	Adverse effects in at least 2% of patients	Outcome	Results/Comments
Double-blind, placebo-controlled, unbalanced parallel-group. To assess safety and efficacy in mild-to-moderate AD disease (36)	15 and 20 t.i.d. orally	491 (164 on placebo)	24 weeks	424 (143 on placebo)	Sinus bradycardia (3.1%), dose-dependent neutropenia (3.1%) granulocytopenia (5.6%), aplastic anemia (one patient)	**ADAS-Cog**: 1.63 points with 15 mg and 2.26 points with 20 mg (significant difference from placebo); **CIBIC-Plus**: 0.33 points with 20 mg (significant difference from placebo); **IADL**: 0.71 points for 20 mg (significant difference from placebo)	Maximal cognitive improvement with 20 mg occurred when RBC AChE inhibition reached 42%.
To assess safety and effectiveness after prolonged treatment in the double-blind, placebo-controlled open-label extension phase of a 25-week mild-to-moderate AD patients (38).	10 t.i.d.	176 89 37	52 w 79 weeks Two years	152 77 33	Transient and generally mild not necessarily drug-related	**ADAS-Cog**: 4.7 (52 w), 6 (79 w), 8.5 (2 years) points vs. corresponding untreated historical patients; **IADL**: 1.7 (52 w), 2.2 (79 w), 3.8 (2 years) points vs. corresponding untreated historical patients	Cognitive benefits translated to about 9 months difference between eptastigmine-treated patients and untreated historical patients.
Double-blind, placebo-controlled, parallel-group study. To assess safety and efficacy in mild-to-moderate AD disease (37)	10 and 12 t.i.d.	342	25 weeks	342 (114 on placebo)	Nausea, vomiting, diarrhea, abdominal pain with similar frequency in drug-and placebo-treated patients.	**ADAS-Cog**: 1.36 points from baseline with 10 and 12 mg; **CDR-SB**: 0.78 from baseline; **MMSE**: 0.21 from baseline.	Level of RBC AChE inhibition directly related to the degree of cognitive improvement in AD patients

Note. w, Based on weight; b.i.d., two times daily; t.i.d., three times daily; IADL, Instrumental Activities of Daily Living Scale; CGIC, Care Giver Impression of Change; LMT, Logical Memory Test; SWFT, Semantic Word Fluency Test; TMT-A, Trail Making Test A; ADAS-Cog, Alzheimer's Disease Assessment Scale–Cognitive Subscale; SBI, Spontaneous Behavior Interview; ADL, Index of independence in Daily Living; BP, Behavioral Problems; CIBIC-Plus, Clinician's Interview-Based Impression of Change Plus; CDR-SB, Clinical Dementia Rating Scale-Sum of the Boxes; MMSE, Mini-Mental State Examination; GDS, Global Deterioration Scale; RBC, red blood cell. Outcome listed statistically significant (at least $p < 0.05$). Adverse events listed are those occurring significantly in at least 2% of patients.

with eptastigmine (10 or 15 mg p.o.) three times daily. Besides the above listed side effects, a reversible granulocytopenia and hyperlipidemia occurred more frequently in patients receiving eptastigmine than placebo. Laboratory variables were not affected except for neutrophil counts which were <1500 per μL in 10 eptastigmine treated patients vs. only one placebo-treated patient (35). Two patients (one receiving eptastigmine, 30 mg t.i.d., and the other 40 mg, t.i.d.) developed reversible neutropenia (33). In another study 5.6% of patients taking 20 mg three times daily for 4 weeks developed granulocytopenia while one patient had an asymptomatic pancytopenia (36).

The mechanism of the toxic hematologic effects is unknown. *In vitro* studies have shown that eptastigmine inhibits the growth of human granulocyte-macrophage colony-forming units from normal peripheral blood progenitor cells in a concentration-dependent fashion (Barosi et al., personal communication, 1992). This effect appears to be linked to the seven-atom aliphatic side chain in the structure of eptastigmine.

Taken together, these findings suggested that the cholinergic tolerability of eptastigmine was found to be favorable, but its potential hematologic effects led to the suspension of further clinical trials (50).

REFERENCES

1. Anderson DJ, Arneric SP. Nicotinic receptor binding of [^{3}H]cytisine, [^{3}H]nicotine and [^{3}H]methylcarbamylcholine in rat brain. *Eur J Pharmacol* 1994;253:261–267.
2. Auteri A, Mosca A, Lattuada N, Luzzana M, Zecca L, Imbimbo BP. Pharmacodynamics and pharmacokinetics of eptastigmine in elderly subjects. *Eur J Clin Pharmacol* 1993;45:373–376.
3. Becker RE, Moriearty P, Unni L. The second generation of cholinesterase inhibitors: Clinical and pharmacological effects. In: Becker RE and Giacobini E, eds. *Cholinergic basis for Alzheimer's therapy.* Boston: Birkhauser, 1991;211–215.
4. Bjornsson TD, Troetel WM, Imbimbo BP. Effect of food of the absorption of eptastigmine. *Eur J Clin Pharmacol* 1998;54:243–247.
5. Braida D. Eptastigmine: A view point. *CNS Drugs* 1998;9:76.
6. Braida D, Ottonello F, Sala M. Eptastigmine improves eight arm radial maze performance in aged rats. *Pharmacol Res* 2000;42:299–303.
7. Braida D, Ottonello F, Sala M. Eptastigmine restores the aged rat's normal cortical spectral power pattern. *Pharmacol Res* 2000;42:495–500.
8. Braida D, Paladini E, Griffini P, Lamperti M, Maggi A, Sala M. An inverted U-shaped curve for heptylphysostigmine on radial maze performance in rats: Comparison with other cholinesterase inhibitors. *Eur J Pharmacol* 1996;302:13–20.
9. Braida D, Sala M. Cannabinoid-induced working memory impairment is reversed by a second cholinesterase inhibitor in rats. *Neuroreport* 2000;11:2025–2029.
10. Braida D, Virag W, Ottonello F, Sala M. Excitatory and inhibitory effects of second-generation cholinesterase inhibitor on rat gastrointestinal transit. *Pharmacol Res* 2000;41:671–677.
11. Brufani M, Castellano C, Marta M, Oliverio A, Pavone F, Pomponi M. National Research Council: European patent No. 85101945.5-1985.
12. Brufani M, Castellano C, Marta M, et al. A long-lasting cholinesterase inhibitor affecting neural and behavioral processes. *Pharmacol Biochem Behav* 1987;26:625–629.
13. Brufani M, Marta M, Pomponi M. Anticholinesterase activity of a new carbamate, heptylphysostigmine, in view of its use in patients with Alzheimer-type dementia. *Eur J Biochem* 1986;157:115–120.
14. Camacho F, Smith CP, Vargas HN, Winslow JT. Alpha$_2$ adrenoceptor antagonists potentiate acetylcholinesterase inhibitor effects on passive avoidance learning in the rat. *Psychopharmacology* 1996;124:347–354.

15. Canal N, Franceschi M. and the Italian eptastigmine Investigators. A double-blind, placebo-controlled, clinical trial in Alzheimer disease patients. In: Giacobini E, Becker R. eds. *Alzheimer disease therapeutic strategies*. Boston: Birkhaeuser, 1994;108–112.
16. Canal N, Imbimbo BP. Eptastigmine Study Group. Relationship between pharmacodynamic activity and cognitive effects of eptastigmine in patients with Alzheimer's disease. *Clin Pharmacol Ther* 1996;60: 218–228.
17. Cattaneo L, Bondiolotti GP, Muller EE, Cocchi D. Effect of acute and short-term administration of cholinomimetic drugs on corticosterone secretion in the rat. *Eur J Pharmacol* 1993;2–3:245–248.
18. Cazzola E, Lattuada N, Zecca L, et al. A rapid potentiometric determination of cholinesterase in plasma and red cells: Application to eptastigmine monitoring. *Chem Biol Interactions* 1993;87:265–268.
19. Cella SG, Imbimbo BP, Pieretti F, Muller EE. Eptastigmine augments basal and GHRH-stimulated growth hormone release in young and old dogs. *Life Sci* 1993;5:389–395.
20. Cuadra G, Giacobini E. Coadministration of cholinesterase inhibitors and idazoxan: Effects of neurotransmitters in rat cortex *in vivo*. *J Pharmacol Exp Ther* 1995;273:230–240.
21. Cuadra G, Summers K, Giacobini E. Cholinesterase inhibitor effects on neurotransmitters in rat cortex *in vivo*. *J Pharmacol Exp Ther* 1994;270:277–284.
22. Cutler NR, Sramek JJ. Target population in phase-I clinical-trials of cholinergic compounds in Alzheimer's disease. The role of the bridging study. *Alzheimer Dis Assoc Disord* 1995;9:139–135.
23. Dawson GR, Bentley G, Draper F, Rycroft W, Iversen SD, Pagella PG. The behavioral effects of heptyl physostigmine, a new cholinesterase inhibitor, in tests of long-term and working memory in rodents. *Pharmacol Biochem Behav* 1991;39:865–871.
24. Dawson GR, Iversen SD. The effects of novel cholinesterase inhibitors and selective muscarinic receptor agonists in tests of reference and working memory. *Behav Brain Res* 1993;57:143–153.
25. De Sarno P, Pomponi M, Giacobini E, Tang XC, Williams E. The effect of heptylphysostigmine, a new cholinesterase inhibitor, on the central cholinergic system of the rat. *Neurochem Res* 1989;14:971–977.
26. Farlow M, Gracon SI, Hershey LA, Lewis KW, Sadowski CH, Dolan-Ureno JA. Controlled trial of tacrine in Alzheimer's disease. *J Am Med Ass* 1992;268:2523–2529.
27. Garrone B, Luparini MR, Tolu L, Magnani M, Landolfi C, Milanese C. Effect of the subchronic treatment with the acetylcholinesterase inhibitor heptastigmine on central cholinergic transmission and memory impairment in aged rats. *Neurosci Lett* 1998;245:53–57.
28. Giacobini E. From molecular structure to Alzheimer therapy. *Jpn J Pharmacol* 1997;74:225–241.
29. Giacobini E. Cholinesterase inhibitors for Alzheimer's disease therapy: From tacrine to future applications. *Neurochem Int* 1998;32:413–419.
30. Giacobini E. Cholinergic foundation of Alzheimer's disease therapy. *J Physiol (Paris)* 1998;92:283–287.
31. Giubilei F., Imbimbo BP, Tisei P. et al. Power spectral analysis of heart rate variability in Alzheimer's disease patients treated with cholinesterase inhibitor. *Neurology* 1996;46(Suppl S):3074.
32. Iijima S, Greig NH, Garofalo P, et al. The long-acting cholinesterase inhibitor heptylphysostigmine attenuates the scopolamine-induced learning impairment of rats in a 14-unit T-maze. *Neurosci Lett* 1992;144: 79–83.
33. Imbimbo BP. Eptastigmine: A cholinergic approach to the treatment of Alzheimer's disease. In: Becker R., Giacobini E, eds. *Alzheimer's disease: From molecular biology to therapy*. Boston: Birkhauser, 1996; 223–230.
34. Imbimbo BP, Licini M, Schettino M. et al. Relationship between pharmacokinetics and pharmacodynamics of eptastigmine in young healthy volunteers. *J Clin Pharmacol* 1995;35:285–290.
35. Imbimbo BP, Lucca U, Lucchelli F, Alberoni M, Thal LJ. A 25-week placebo-controlled study of eptastigmine in patients with Alzheimer disease. *Alzheimer Dis Assoc Disord* 1998;12:312–322.
36. Imbimbo BP, Martelli P, Troetel WM, et al. Efficacy and safety of eptastigmine for the treatment of patients with Alzheimer's disease. *Neurology* 1999;52:700–708.
37. Imbimbo BP, Troetel WM, Martelli P., Lucchelli F. and the Eptastigmine Study Group. A 6-month, double-blind, placebo-controlled trial of eptastigmine in Alzheimer's disease. *Dement Geriatr Cogn Disord* 2000; 11:17–24.
38. Imbimbo BP, Verdelli G., Martelli P., Marchesini D. Two-year treatment of Alzheimer's disease with eptastigmine. *Dement Geriatr Cogn Disord* 1999;10:139–147.
39. Iversen LL. Approaches to cholinergic therapy in Alzheimer's disease. *Prog Brain Res* 1993;98:423–426.

40. Lines CR, Ambrose JH, Heald A, Traub M. A double-blind, placebo-controlled study of the effects of eptastigmine on scopolamine-induced cognitive deficits in healthy male-subjects. *Hum Psychopharm Clin* 1993; 8:271–278.
41. Linville DG, Giacobini E, Arneric SP. Heptyl-physostigmine enhances basal forebrain control of cortical cerebral blood flow. *J Neurosci Res* 1992;31:573–577.
42. Liu WF. Effects of cholinesterase inhibitors on a two-component chained schedule performance. *Neurotoxicol Teratol* 2000;22:389–396.
43. Mant T, Troetel WM, Imbimbo BP. Maximum tolerated dose and pharmacodynamics of eptastigmine in elderly healthy volunteers. *J Clin Pharmacol* 1998;38:610–617.
44. Mayeux R, Sano M. Drug therapy: Treatment of Alzheimer's disease. *New Engl J Med* 1999;341: 1670–1679.
45. McClellan K, and Benfield P. Eptastigmine. *CNS Drugs* 1998;9:69–75
46. Mori F, Lai C-C, Fusi F, Giacobini E. Cholinesterase inhibitors increase secretion of APPs in rat brain cortex. *Neuroreport* 1995;6:633–636.
47. Moriearty PL. Transdermal delivery of cholinesterase-inhibitors. Rationale and therapeutic potential. *CNS Drugs* 1995;4:323–334.
48. Moriearty PL, Becker RE. Inhibition of human brain and RBC acetylcholinesterase (AChE) by heptylphysostigmine (HPTL). *Meth Find Exp Clin Pharmacol* 1992;14:615–621.
49. Mosca A, Onelli E, Rosti E, Paleari M, Luzzana M, and Imbimbo BP. A patient-side technique for real-time measurement of acetylcholinesterase activity during monitoring of eptastigmine treatment. *Ther Drug Monit* 1995;17:230–238.
50. Nordberg A, Svensson AL. Cholinesterase inhibitors in the treatment of Alzheimer's disease: A comparison of tolerability and pharmacology. *Drug Safety* 1998;19:465–480.
51. Ogane N, Giacobini E, Messamore E. Preferential inhibition of acetylcholinesterase molecular forms in rat brain. *Neurochem Res* 1992;17:489–495.
52. Ogane N. Giacobini E, Struble R. Differential inhibition of acetylcholinesterase molecular forms in normal and Alzheimer disease brain. *Brain Res* 1992;589:307–312.
53. Perini M, Montanini R, Casucci R. Effects of eptastigmine, a new cholinesterase inhibitor, on regional cerebral blood flow in Alzheimer patients. *J Neurol* 1995(Suppl 2):242.
54. Rupniak NMJ. Profile of cholinomimetic drugs in primates-status of screens for potential Alzheimer therapies. *Drug Dev Res* 1992;27:77–88.
55. Rupniak NMJ, Tye SJ, Brazell C, Heald A, Iversen SD, Pagella PG. Reversal of cognitive impairment by heptyl-physostigmine, a long-lasting cholinesterase inhibitor, in primates. *J Neurol Sci* 1992;107:246–249.
56. Scremin OU, Heuser D, Hudgell R, Romero E, Imbimbo BP. Prolonged effects of cholinesterase inhibition with eptastigmine on the cerebral blood flow-metabolism ratio of normal rats. *J Cerebr Blood Flow Metab* 1993;13:702–711.
57. Scremin OU, Li MG, Scremin AME, Jenden DJ. Cholinesterase inhibition improves blood flow in the ischemic cerebral cortex. *Brain Res Bull* 1997;42:59–70.
58. Segre G, Cerretani D, Baldi A, Urso R. Pharmacokinetics of heptastigmine in rats. *Pharmacol Res* 1992; 25:139–146.
59. Sramek JJ, Block GA, Reines SA et al. A multiple-dose safety trial of eptastigmine in Alzheimer's desease, with pharmacodynamic observations of red blood cell cholinesterase. *Life Sci* 1994;56:319–326.
60. Tabano MT, Luzi M, Palazzesi S, Pomponi M, Loizzo A. Effects of cholinergic drugs on neocortical EEG and flash visual evoked potentials in the mouse. *Neuropsychobiology* 1999;40:47–56.
61. Tuovinen K, Kalistekorhonen E, Raushel FM, Hanninen O. Eptastigmine-phosphotriesterase combination in DFP intoxication. *Toxicol Appl Pharm* 1996;140:364–369.
62. Tuovinen K, Hanninen O. Protection of mice against soman by pretreatment with eptastigmine and physostigmine. *Toxicology* 1999;139:233–241.
63. Tuovinen K, Kalistekorhonen E, Raushel FM, Hanninen O. Phosphotriesterase pralidoxime-2-chloride (2-PAM) and eptastigmine treatments and their combinations in DFP intoxication. *Toxicol Appl Pharm* 1996;141:555–560.
64. Tuovinen K, Kalistekorhonen E, Raushel FM, Hanninen O. Success of pyridoxstigmine, physostigmine, eptastigmine and phosphotriesterase treatments in acute sarin intoxication. *Toxicology* 1999;134:169–178.
65. Unni LK, Becker RE. Determination of heptylphysostigmine by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 1992;573:275–281.

66. Unni LK, Hutt V, Imbimbo BP et al., Kinetics of cholinesterase inhibition by eptastigmine in man. *Eur J Clin Pharmacol* 1991;41:83–84
67. Waite JJ, Thal LJ. The behavioral effects of heptylphysostigmine on rats lesioned in the nucleus accumbens. *Neurosci Res* 1995;21:251–259.
68. Winslow JT, Camacho F. Cholinergic modulation of a decrement in social investigation following repeated contacts between mice. *Psychopharmacology* 1995;121:164–172.
69. Zecca L, Radice D, Mosca A, Pagella PG. Determination of heptylphysostigmine in plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatogr Biomed Appl* 1993;615: 169–173.
70. Zhu XD, Cuadra G, Brufani M, et al. Effects of MF268, a new cholinesterase inhibitor, on acetylcholine and biogenic amines in rat cortex. *J Neurosci Res* 1996;43:120–126.