Pharmaceutical Research, Vol. 7, No. 6, 1990

Report

Application of a Brain-Targeting Chemical Delivery System to 9-Amino-1,2,3,4-Tetrahydroacridine[1]

Emil Pop,[2,3] Katalin Prókai-Tátrai,[2,3] James D. Scott,[3] Marcus E. Brewster,[2,3] and Nicholas Bodor[2–4]

Received August 28, 1989; accepted December 15, 1989

Several chemical delivery systems (CDS) were synthesized for the cholinesterase inhibitor 9-amino-1,2,3,4-tetrahydroacridine (THA). The derivatives prepared were substituted with a 1,4-dihydropyridine ⇌ pyridinium salt redox system at the amino functionality. These compounds were synthesized by acylation of the 9 amino group of THA with nicotinic anhydride under forced conditions, followed by a selective N-alkylation of the pyridine ring and regioselective reduction of the resulting quaternary salts. Lipophilicity parameters indicated increased lipophilic indices for various CDS's compared to the THA. Oxidation studies showed that dihydronicotinamides readily converted to the quaternary salt, both chemically and enzymatically. The transport forms of THA were also shown not to interact with acetylcholinesterase *in vivo*. *In vivo* distribution studies in the rat indicated that high and sustained levels of the pyridinium quaternary ion derivative were present in the central nervous system (CNS). In addition, THA was produced in the CNS from the quaternary salt precursor in low concentrations, indicating a slow but sustained release. The CDS for THA were found to be less acutely toxic than THA.

KEY WORDS: tetrahydroacridine; Alzheimer's disease; chemical delivery system.

INTRODUCTION

Senile dementia of the Alzheimer type (AD) (1,2) is a slowly progressive neuropsychiatric condition that is principally manifested by memory deficits and culminates in the death of the individual (3). This disease which affects older people is clearly a major public health problem (4,5).

The causes of AD are, as of now, uncertain. One theory which has received considerable attention is that AD is in some way related to decrements in the central cholinergic system (6–9). This hypothesis is rooted in the observations that cholinergic neurons are severely depleted in AD patients and that this neuronal loss in experimental circumstances can induce memory loss consistent with that seen in AD (10,11). Loss of memory is the most reliable symptom occurring in individuals stricken with AD. In addition, acetylcholine synthesis, choline acetyltransferase activity, acetylcholinesterase activity, and choline uptake are all markedly reduced in patients diagnosed as having AD (12). Finally, the severity of the dementia has been correlated with a cholinergic activity and the diminution of choline in the cerebrospinal fluid (13).

Rational approaches to AD therapy have therefore been based on attempts to improve cholinergic functioning by using cholinomimetic drugs which could potentially reverse memory loss. The following regimens have been examined: precursor loading, administration of muscarinic and nicotinic cholinergic agonists, cholinesterase inhibition, and a combination of precursor loading and cholinesterase inhibition. As precursors of acetylcholine, choline and lecithin have been administered (14). Unfortunately no convincing evidence for stimulation of acetylcholine synthesis was reported. Results from the administration of agonist drugs have varied: systemic administration was unsuccessful but direct administration of bethanechol into CSF improved both memory and functional competence in daily living activities (15). However, the effects are highly variable. Oral administration of physostigmine, a potent cholinesterase inhibitor, has not resulted in consistent improvement of memory or daily living functions (16). A large number of studies have examined the administration of physostigmine by oral, intravenous, or intramuscular routes under either acute or chronic double-blind conditions but results have been disappointing. The combination of physostigmine or other cholinomimetic drugs with the acetylcholinesterase precursor, choline, given as such or as lecithin does not appear to be efficacious. The use of metrifonate, which does not act as an inhibitor of cholinesterases directly but requires a chemical transformation to 2,2-dichlorovinyldimethylphosphate, has been shown to be useful in AD (6). The best results thus far obtained have unquestionably been in a recent clinical study

---

[1] Contribution No. 49 in the series "Improved Delivery Through Biological Membranes."

[2] Center for Drug Design and Delivery, College of Pharmacy, University of Florida, Gainesville, Florida 32610.

[3] Pharmatec, Inc., Alachua, Florida 32615.

[4] To whom correspondence should be addressed.

with 9-amino-1,2,3,4-tetrahydroacridine (THA; tacrine), a weak cholinesterase inhibitor (17).

Based on this study THA was shown to alleviate the symptoms of AD. Encouraged by these results, a multicenter clinical trial to test THA was begun. After being temporarily suspended because of signs of liver toxicity and damage (18) the trial has been recently continued. Derivatives of THA, claiming lower toxicity, such as 9-amino-1,2,3,4-tetrahydroacridine-1-ol, have been recently reported (19).

A method that would deliver THA selectively to the CNS, lowering the possibility of peripheral, toxic effects would be beneficial. The application of the brain-targeting chemical delivery system (CDS) to THA could offer a solution of this problem. The CDS is based on conversion of a drug into a derivative that contains various molecular carriers, such as 1-alkyl-1,4-dihydronicotinates, 1-alkyl-1,4-dihydronicotinamides, or other 1-alkyl-1,4-dihydronicotinic acid derivatives. Because of the enhanced lipophilicity of these conjugates, systemic administration of derivatized drug yields an extensive tissue distribution, the drug partitioning into both the CNS and the periphery. The dihydropyridine derivative undergoes enzymatic oxidation to give the corresponding quaternary pyridinium salt form. This now polar molecule is sequestered in the brain behind the lipoidal blood–brain barrier (BBB), while it is readily eliminated from the periphery. With time the "locked-in" species degrades by hydrolysis to yield the parent drug, which can subsequently exert its pharmacologic action. The small, polar carrier is readily lost from the CNS in active processes. The system permits the penetration of polar, hydrophilic drugs through the BBB into the CNS, allowing for a more prolonged delivery and reduces peripheral levels of the drug thus attenuating non-target site toxicities. The principle of the CDS has been reviewed (20–32). The CDS approach has been successfully applied to many drugs such as dopamine, γ-aminobutyric acid and tryptamine (20–22), sex steroids (23–25), glucocorticoids (26), antiepilectic agents (27), anticancer agents (28,29), antibiotics (30,31), antidepressants (32), and others.

MATERIALS AND METHODS

Chemistry

The compounds synthesized were characterized by melting points, elemental analysis, ultraviolet (UV), proton nuclear magnetic resonance (^{1}H-NMR), and mass spectrometry (MS). Uncorrected melting points determined by Electrothermal melting-point apparatus (Fischer Scientific) are reported. Elemental microcombustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, Georgia. Ultraviolet spectra were determined in methanol using a Hewlett Packard 8451A diode array spectrophotometer. ^{1}H-NMR were recorded on a Varian XL 300 MH_z (300 FT mode) spectrometer. Samples were dissolved in deuterated dimethylsulfoxide (DMSO-d_6) and chemical shifts were reported as parts per million (δ) relative to tetramethylsilan as an internal standard. Coupling constants (J) are reported in Hertz. Mass spectra were recorded on a Kratos, MS 80-RFA instrument. Fast atom bombardment (FAB) ionization was performed by xenon beam (6 keV) and dissolving the samples in glycerol, as described in literature (33). All chemicals were reagent grade. THA was obtained from Aldrich Chemical Co. Inc.

N-*(1,2,3,4-Tetrahydro-9-acridinyl)nicotinamide (2).* A mixture of 1.98 g (10 mmol) THA (1) and 2.51 g (11 mmol) nicotinic anhydride in 50 ml dry pyridine was refluxed for 48 hr. The resultant solution was poured into 500 g ice water and the solid filtered off, washed with water, and dried. The crude material was dissolved in methanol and precipitated with ether. 2 was isolated as an off-white substance with 75% yield (2.3 g), MP 232–234°C. ^{1}H-NMR: δ 1.78–1.91 (m, 4H, THA C-3 and C-2 protons); 2.85 (t, 2H, J = 6.20, THA C-1 proton); 3.09 (t, 2H, J = 6.10, THA C-4 proton); 7.49–7.71 (m, 3H, THA, C-6, C-7 and pyridine C-5 protons); 7.93–7.97 (m, 2H, THA C-8, pyridine C-4 protons); 8.45 (d, 1H, J = 7.92, THA C-5 proton); 8.85 (d, 1H, J = 5.75, pyridine C-6 proton); 9.30 (s, 1H, pyridine C-2 proton); 10.67 (s, 1H, NH). MS (FAB): $[M + H]^+$ = m/z 304. Anal calcd. for $C_{19}H_{17}H_{30}$: C, 75.23; H, 5.60; N, 13.84; Found: C, 75.20; H, 5.69; N, 13.84.

*1-Methyl-3-[(1,2,3,4-tetrahydro-9-acridinyl)carbamoyl] pyridinium-*p*-toluenesulfonate (3a).* A solution of 1.52 g (5 mmol) of 2 and 0.93 g (5 mmol) of methyl-*p*-toluenesulfonate in 25 ml of acetonitrile and 5 ml of methanol was heated at 50–60°C for 4 days. The solvent was removed *in vacuo,* and the residue slurried with ether, filtered, and dried. The crude material was recrystallized from methanol:ether, 2.4 g (yield 98%) of 3a resulting as a hygroscopic solid. MP 238–242°C. UV max: 222, 312, 324, 358 nm. ^{1}H-NMR: δ 1.78–1.91 (m, 4H); 2.24 (s, 3H, Ts-CH_3); 2.83 (t, 2H, J = 6.73); 3.14 (t, 2H, J = 6.74); 4.48 (s, 3H, N^+-CH_3); 8.3–7.02 (m, 9H, THA, Ts aromatic and pyridinium C-5 proton); 9.91–9.44 (m, 2H, pyridinium C-6 and C-4 protons); 9.63 (s, 1H). MS (FAV): C^+ = m/z 318. Anal calcd for $C_{27}H_{27}N_3O_4S$: C, 66.24; H, 5.56; N, 8.58. Found: C, 66.11; H, 5.59; N, 8.61.

1-Propyl-3-[(1,2,3,4-tetrahydro-9-acridinyl)carbamoyl] pyridinium Iodide (3b). A solution of 1.52 g (5 mmol) of 2 and 0.88 g (5 mmol) of 1-iodopropane in 25 ml of acetonitrile and 5 ml of methanol was stirred at 50°C for 3 days. The solvent was removed *in vacuo* and the residue slurried with ether. After recrystallization from methanol-ether, 2.3 g of 3b (yield, 93%) was obtained as a yellow solid. MP 105–108°C (dec). UV max: 227, 310, 324 nm. ^{1}H-NMR: δ 0.98 (t, 3H, J = 7.32, N^+CH_2-CH_2-CH_3); 1.78–2.14 (m, 6H, THA C-3, C-2 protons, N^+-CH_2-CH_2-CH_3; 2.94 (t, 2H,J = 5.07); 3.29 (t, 2H, J = 5.07); 4.81 (t, 2H, J = 7.47, N-CH_2-CH_2-CH_3); 7.72–8.80 (m, 5H, THA aromatic and pyridinium C-7 proton); 9.21–9.26 (m, 1H, pyridinium C-4 proton); 5.46 (d, 1H, J = 5.96, pyridinium C-6 proton); 9.83 (2, 1H, pyridinium C-2 proton). MS (FAB): C^+ = m/z 346. Anal calcd. for $C_{22}H_{24}IN_3O_3$: C, 55.84; H, 5.11; I, 26.82; N, 8.88. Found: C, 55.59; H, 5.17; I, 26.70; N, 8.71.

1-Benzyl-3-[(1,2,3,4-tetrahydro-9-acridinyl)carbamoyl] pyridinium Bromide (3c). In the same way from 1.52 g (5 mmol) 2 and 0.88 g (5 mmol) benzyl bromide in 25 ml acetonitrile and 5 ml methanol in 2 days at 40°C and recrystallization from methanol–ether, 2.2 g (93%) of 3c resulted in a yellow solid. MP 220–224°C. UV max: 216, 232, 310, 324 nm. ^{1}H-NMR δ: 1.75–1.88 (m, 4H); 2.91–3.09 (m, 4H); 6.11 (s, 2H, C_6H_5-CH_2); 7.47–8.42 (m, 10H); 9.34 (d, 1H, J = 7.52); 9.49 (d, 1H, J = 4.80); 10.05 (s, 1H) MS (FAB): C^+ = m/z

395. Anal calcd. for $C_{26}H_{24}BrN_3O_4$: C, 65.80; H, 5.06; Br, 16.85; N, 8.86. Found: C, 65.68; H, 5.11; Br, 16.83; N, 8.82.

*1-(*p*-Nitrobenzyl)-3-[(1,2,3,4-tetrahydro-9-acridinyl)carbamoyl]pyridinium Bromide* (**3d**). Following the same procedure from 1.52 g (5 mmol) **2** and 1.08 g (5 mmol) *p*-nitrobenzyl bromide in 25 ml acetonitrile and 5 ml methanol in **3d** at 50°C and recrystallization from methanol–ether, 2.4 g (92%) of **3d** resulted in a yellow solid. MP 190–195°C. UV max: 213, 269 nm. ^{1}H-NMR δ: 1.82–1.92 (m, 4H); 2.93–3.14 (m, 4H); 6.30 (s, 2H); 7.55–8.51 (m, 9H); 9.39 (d, 1H, J = 7.9); 9.55 (d, 1H, J = 5.62); 10.11 (s, 1H). MS (FAB) C^+ = m/z 440. Anal calcd. for $C_{26}H_{23}BrN_3O_3$: C, 60.16; H, 4.42; Br, 15.38; N, 10.29. Found: C, 59.81; H, 4.57, Br, 15.61; N, 10.37.

*1,4-Dihydro-1-methyl-*N*-(1,2,3,4-tetrahydro-9-acridinyl) nicotinamide* (**4a**). To a solution of 1.40 g (1 mmol) of **3a** in deaerated water, a mixture of 0.35 g (4 mmol) sodium bicarbonate and 0.7 g (4 mmol) sodium dithionite was added. The mixture was stirred at 20–25°C for 1 hr, and the precipitated solid material was filtered off and rinsed with deaerated, cold water. After drying in inert atmosphere 0.2 g (64%) of **4a** resulted as a yellow solid. MP 185–190°C. UV max: 218, 298, 304, 314, 368 nm. ^{1}H-NMR: δ 1.76–1.87 (m, 4H); 2.73 (t, 2H, J = 6.38); 2.95 (s, 3H, N-CH_3); 3.02 (t, 2H, J = 6.30); 3.18 (bs, 1H, pyridine C-4 protons); 4.74–4.71 (m, 1H, pyridine C-5 proton); 5.90 (d, 1H, J = 9.39, pyridine C-6 proton); 7.13 (s, 1H, pyridine C-2 proton); 7.45–7.88 (m, 4H); 9.01 (s, 1H, NH). Anal calcd. for $C_{20}H_{21}N_3O.1.5\,H_2O$: C, 69.34; H, 6.98; N, 12.13 Found: C, 69.71; H, 6.75; N, 12.13.

*1,4-Dihydro-1-propyl-*N*-(1,2,3,4-tetrahydro-9-acridinyl) nicotinamide* (**4b**). Following the same procedure from 0.47 g (1 mmol) **3b** in 30 ml water reduced with 0.52 g (5 mmol) $NaHCO_3$ and 0.7 g (4 mmol) $Na_2S_2O_4$ for 20 min, 0.2 g (58%) **4b** resulted as a yellow solid. MP 165–170°C. UV max: 228, 296, 324, 339, 340 nm. ^{1}H-NMR: 1.09 (t, 3H, J = 6.99); 1.52–1.99 (m, 6H); 2.95 (t, 2H, J = 3.50); 2.97–3.35 (m, 6H); 4.63–4.73 (m, 1H); 5.98 (d, 1H, J = 1.11); 7.17 (s, 1H); 7.16–8.88 (m, 4H). Anal calcd. for $C_{22}H_{25}N_3O.1.5\,H_2O$: C, 70.92; H, 7.52; N, 11.25. Found: C, 71.03; H, 7.83; N, 11.38.

*1-Benzyl-1,4-dihydro-*N*-(1,2,3,4-tetrahydro-9-acridinyl) nicotinamide* (**4c**). By reducing 0.48 g (1 mmol) of **3c** in 45 ml water and 45 ml EtOAc with 0.52 g (6 mmol) $NaHCO_3$ and 0.71 g (4 mmol) $Na_2S_2O_4$ in 2 hr followed by separation of layers, extraction of the aqueous one with EtOH, then drying on Na_2SO_4 and concentrating the organics to dryness 0.22 g (54%) of **4c** resulted. MP 158–162°C. UV max: 224, 228, 235, 240, 324 nm. ^{1}H-NMR δ: 1.76–1.87 (m, 4H); 2.73 (t, 2H, J = 6.30); 2.09 (t, 2H, J = 6.35); 3.24 (s, 2H, C_6H_5-CH_2); 3.37 (bs, 2H); 4.74–4.77 (m, 1H); 5.95–6.01 (m, 1H); 7.28–7.88 (m, 10H); 9.01 (s, 1H). Anal calcd. for $C_{26}H_{25}N_3O.0.5\,H_2O$: C, 77.13; H, 6.12; N, 10.38. Found: C, 77.39; H, 6.51; H, 10.45.

*1,4-Dihydro-1-(*p*-nitrobenzyl)-*N*-(1,2,3,4-tetrahydro-9-acridinyl)nicotinamide* (**4d**). By reducing in the same way 0.52 g (1 mmol) **3d** in 40 ml water–40 ml EtOAc with 0.52 g (6 mmol) $NaHCO_3$ and 0.71 g (4 mmol) $Na_2S_2O_4$ in 3 hr, 0.24 g (56%) **4d** resulted in a yellow solid. MP 156–160°C. UV max: 211, 365 nm. ^{1}H-NMR: 1.81–1.89 (m, 4H); 2.82 (t, 2H, J = 6.20); 3.07 (t, 2H, J = 6.20); 3.16 (s, 2H); 3.42 (bs, 2H); 4.10–4.14 (m, 1H); 6.68–6.70 (m, 1H); 7.50–8.42 (m, 9H); 9.25 (s, 1H) Anal calcd. for $C_{26}H_{24}N_4O_3.0.5\,H_2O$: C, 69.40; H, 5.56; N, 12.45 Found: C, 69.75; H, 5.79; N, 12.17.

Chemical Oxidation Studies

The rate of ferricyanide-mediated oxidation of the THA-CDSs was determined using a modification of published methods (34). In this procedure, the rate of decrease of the 358-nm absorbance band of the compounds of interest was determined in buffered 20% aqueous acetonitrile solutions [0.1 m*M* $K_4Fe(CN)_6$, 60 m*M* KCl, and 1.0 m*M* K_2CO_3] containing various concentrations of $K_3Fe(CN)_6$ (1–50 m*M*). The dihydronicotinamides in acetonitrile were added, using a Hamilton syringe, to the test solutions, which were maintained at 37°C in a thermostated cell holder and contained in anaerobic screw-top cuvettes (Spectrocell, Inc.), blanketed with argon. For a given $Fe(CN)_6{}^{-3}$ concentration, the pseudo-first-order rate constant was determined, and then these values were plotted as a function of ferricyanide ion concentration, generating a slope from which the second-order (k; $sec^{-1}\ M^{-1}$) was obtained.

Lipophilicity Measurements: Determination of R_m Values

A measurement of lipophilicity of compounds of interest was carried out chromatographically on TLC plates, Baker, Si-C18F 19C, 20 × 20 glass plates precoated with octadecysilane (C18) reversed-phase bonded to silica gel, app. 20-μm particle size, 200-μm hard surface layer with 254-nm fluorescent indicator and 19 channels each of 9-mm width. The compounds were dissolved in acetone and 1 μl of a 3 mg/ml solution was applied onto each channel along a line 2 cm above the bottom of the plate in random locations. The mobile phase of 100 ml of water or various concentrations of acetone in water and the solvent was eluted 14 cm from the origin. The developed plates were dried and the compounds were visualized by ultraviolet light. The corresponding R_m values were calculated from the R_f values by means of the equation $R_m = \log(1/R_f - 1)$. The theoretical values of 100% water in mobile phase were calculated by the least-squares regression method from the R_m values as a function of water content in the mobile phase.

Analytical Method

A high-performance liquid chromatography (HPLC) method was developed for quantitative analysis. The HPLC system consisted of a Spectra-Physics SP 8810 pump, an SP8780 autosampler, and SP 4290 integrator, a Kratos 747 spectroflow UV detector (242 nm, THA and quarternary salts) and a Kontron SFM 2318 (fluorescence) detector (310-nm excitation and 360-nm emission, THA). The two detectors were employed in series. The limit of fluorescence detection was 0.0678 μg/ml (0.31 ng injected), compared to 0.0313 μg/ml (1.25 ng injected) for the UV detector. In all determinations a Spherisorb C-8 5μ (Alltech Cat. No. 8744) 250-mm × 4.6-mm-i.d. analytical column, jacketed and maintained 30°C, was used. The mobile phase consisted of acetonitrile: KH_2PO_4, 40 m*M*, pH, 5.4, 35:65, and 0.11 m*M* tetrabutylammonium perchlorate. The flow rate was 1.2 ml/min.

In Vitro Studies

The stability of 4a in acetonitrile (100% HPLC grade), phosphate buffer (154 m*M* K_2HPO_4/H_2O, pH 7.4) and rat tissue homogenates (20% liver, wet weight/vol, in phosphate buffer; 20% brain, wet weight/vol, in phosphate buffer; 50% whole blood, vol/vol, in phosphate buffer) was determined. Freshly collected blood and tissues were homogenized in the chilled pH 7.4 phosphate buffer on ice. To 2-ml aliquots of homogenates and control solution, prewarmed at 37°C (except for CH_3CN, room temp.), 20 μl of 4a stock solution (1.6 mg/ml 4a in acetonitrile, 5×10^{-3} *M*) was added (time zero), then samples (100 μl) were taken at appropriate time intervals from 30 sec to 120 min and added to 600 μl acetonitrile. After centrifuging, supernatants were removed and analyzed by HPLC. Each sampling was performed in duplicate. Standards of 4a were prepared from the same 5×10^{-3} *M* stock solution and used to calculate the recovery from the matrices; it was found that 88% of CDS was recovered from supernatants. Pseudo-first-order rate constants for the disappearance of compounds in biological media were determined by linear regression analysis from plots of log peak area versus time. Reactions in biological media were followed for three half-lives and no nonlinearity was observed later in the course of the reaction.

In Vivo Distribution Studies

Male Sprague–Dawley rats (Harlan, BW, 250 g) were used. The distribution of 4a was studied in the rat. A limited dose ranging experiment was conducted to determine the amount of 4a to be administered. Equimolar doses of THA and 4a were administered. The toxicity of the THA was considerably higher compared to that of 4a. While doses up to 45 mg/kg of 4a were tolerated, only about 1/10 of the respective molar equivalent (2.8 mg/kg) could be used safely in the case of THA. The distribution studies were performed by using 2.8 mg/kg THA and 30 mg/kg 4a, both in 0.5 ml DMSO used as a vehicle. DMSO controls (0.5 ml/kg were included). The THA and 4a were administered in the tail vein of conscious restrained animals. Animals were sacrificed at different time intervals following administration: 15 min, 30 min, 1 hr, 2 hr, 6 hr, and 24 hr for 4a and DMSO and 30 min, 2 hr, and 6 hr for THA control. Three animals were used for each time point. Animals were decapitated and trunk blood was collected in heparinated tubes. Brain and liver were removed, weighed, and frozen on dry ice. In preparing for analyses, each organ was homogenized in isotonic phosphate buffered saline to give a 20% (w/v) concentration. Blood was diluted 1:1 with buffer. The homogenates were deproteinized and extracted with acetonitrile (1 ml homogenate mixed with 2 ml acetonitrile), vortexed, and centrifuged 3 min (13,000 rpm, Beckman Microfuge 12). The upper organic layer was carefully taken off, placed in autosampler vials, and stored at −70°C until analyzed. The CDS, THA, and different metabolites (quaternary salt, water addition products) were determined by HPLC.

RESULTS AND DISCUSSION

Synthesis

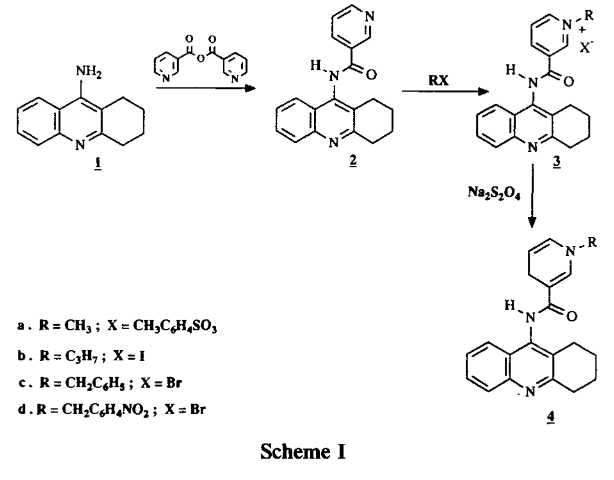
Several potential brain-targeting derivatives of THA were synthesized according to Scheme I. All of the CDSs contained a nicotinamide group attached to the 9-amino function and each was distinctively alkylated at the pyridine ring nitrogen. THA is unusually stabile due to electronic delocalization with the result that the amino group is almost unreactive (35). The only *N*-acylated derivative of THA reported so far is the 9-acetamide (36). The direct 9-*N*-alkylation of THA seems to be impossible since all *N*-alkyl compounds thus far prepared have been synthesized by indirect methods from 9-halo-1,2,3,4-tetrahydroacridine and a primary amine. By using forced conditions, however, THA was acylated with nicotinic anhydride in refluxing pyridine. Another method of *N*-acylation can be by deprotonation of the 9-amino group with metal hydrides or bases (sodium hydride, potassium t-butoxide, *n*-butyl lithium, etc.) and reacting the resulted salts with nicotinoyl chloride.

The possibility of nonselective alkylation on both the pyridine and the tetrahydroacridine ring nitrogen atoms was a potential problem in the next step of the synthesis. MO calculation (35) suggested that electronic, thermodynamic, and steric factors could control this reaction. Based on the findings that the pyridine nitrogen is less hindered than that of THA, where the twisted saturated ring may present an obstacle for the approaching alkylating agents, bulky alkylating agents, such as methyl-*p*-toluene sulfonate, propyl iodide, benzyl, or nitrobenzyl bromide, were employed. Under these conditions, the pyridine *N*-alkylated quaternary salts 3 could be selectively obtained. The reduction of 3 was performed with sodium dithionite to give regioselective reduction, yielding the 1,4-dihydropyridine isomers (37). All compounds were characterized by elemental analysis, UV, ^{1}H-NMR spectroscopy, and mass spectrometry. Chromatographic techniques (HPLC, TLC) indicated the presence of only one component in each case. The dihydropyridine derivatives were shown to be the 1,4 isomers by their UV maxima at ~360 nm and ^{1}H-NMR absorbances.

Redox Properties

All dihydropyridine derivatives could be quantitatively converted to the corresponding pyridinium salts by the action of methanolic silver nitrate solution or H_2O_2 in the presence of catalytic cupric ions.

The rate of potassium ferricyanide-mediated oxidation



Scheme I

was determined for 4a in order to assess the oxidation stability of a THA CDS and compare it to other redox systems. The oxidation was followed spectrophotometrically by standard methods (34,38). The second-order rate constant for ferricyanide-mediated oxidation of 4a is 52.25 $\sec^{-1} M^{-1}$, which correlates empirically with successful CDS operation. The reactivity of this species is not such that handling and storage at the CDS would cause significant degradation. The nature of the oxidation of 4a suggests that the CDS is stable enough to penetrate the CNS and, once in the brain or CSF, rapidly to oxidize to the corresponding pyridinium salt, effectively trapping the CDS.

Lipophilicity

Lipophilicity is an important factor in controlling the interaction of drugs with biological systems. Significant lipophilicity is essential for a CDS to penetrate different barrier systems of the brain. THA is a relatively lipoidal molecular that is able to cross the BBB. Chromatographic R_m values were used to assess the lipophilicity of different CDS's relative to THA. It is known that R_m is related to the partition coefficient between the mobile and stationary phase of a chromatographic system and is correlated with the penetration of substances in biological cells (39–41) and as such is a useful index of lipophilicity. The R_m values (Table I) were calculated from the relation $R_m = \log(1/R_f - 1)$, where the R_f were measured by means of a reversed-phased TLC method with various concentrations of acetone and water in the mobile phase. By extrapolation of the linear part of R_m value curves to a mobile phase of 100% water, the lipophilic indices (extrapolated R_m values) were calculated (Table II). The CDSs were, as expected, more lipophilic than the parent drug. The lipophilicity increased in the series 1 < 4a < 4b < 4c, proportional with the number of carbons in the substituent at 1 position. The polar nitro group in 4d reduced the lipophilicity of the molecule considerably (from 2.87 to 2.11). Based on R_m values of the CDSs relative to THA, it appears that all of them should be able to penetrate the BBB efficiently.

In Vitro Stability

The stability of the CDS 4a was determined in phosphate buffer at physiological pH 7.4 and in several important biological matrices. The following chemical and/or enzymatical transformations were expected for the CDS: oxidation (to the quaternary salt), hydrolysis (yielding THA), and acid-catalyzed hydration (resulting in 6-hydroxy-1,2,3,4-tetrahydropyridine derivative, 5) (Scheme II).

Table I. R_m Values for THA-CDSs in Different Mobile Phases (Acetone:Water)

Compound	Acetone (%)				
	30	50	60	70	80
THA (1)	0	1.2470	1.1300	1.0210	0.9180
4a	1.2180	0.3017	−0.0938	−0.4438	−0.6550
4b	0	0.6070	0.2010	−0.1680	−0.4600
4c	0	0.8127	0.3170	−0.0960	−0.4480
4d	0	0.2896	−0.1188	−0.5900	−0.7890

Table II. Lipophilic Indexes (Extrapolated R_m Values) for THA-CDSs[a]

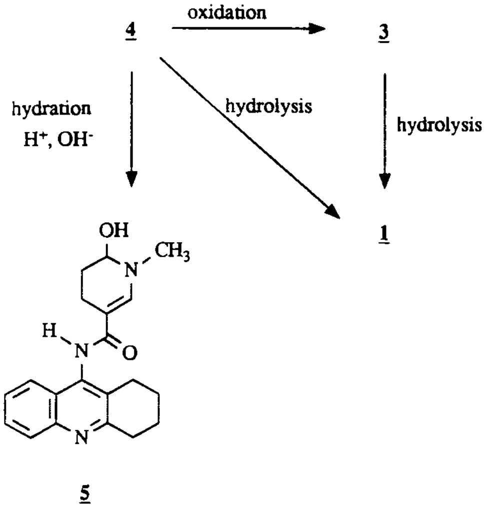
Compound	R_m	r
THA (1)	1.80	−0.999
4a	2.27	−0.990
4b	2.36	−0.997
4c	2.87	−0.997
4d	2.11	−0.988

[a] r = correlation coefficient; $R_m = \log(1/R_f - 1)$.

Table III shows the calculated half-lives for 4a in different media. The data indicate that the stability of the CDS in organic solvents is much higher compared to that in the aqueous media. In phosphate buffer pH 7.4, the disappearance of 4a was rapid. Surprisingly, the main reaction product was the water addition adduct (5a), accompanied by some oxidation product. THA was produced by the hydrolysis of 4a or of the corresponding quaternary salt form. In all biological homogenates, the water addition product appears in low amounts; the main reaction product being the quarternary pyridinium salt. Liver homogenate was the least stable medium for 4a but the degradation product obtained from this matrix did not include the quaternary salt or the water addition product, indicating a different metabolic pathway occurred in this organ homogenate. The stability of 4a in blood is consistent with prior experiences with other CDSs and was the highest among all tested biological materials (over 30 min). Both the quaternary salt and water addition products could be detected in blood after CDS addition. In brain, the main products of degradation were the quaternary salt and the water addition product although some THA was produced. Based on the described oxidation, lipophilicity, and stability studies, 4a and the other THA derivatives present the required physicochemical properties to deliver the drug to the CNS.

In Vitro Activity Studies

One of the tenents of the CDS approach is that deriva-



Scheme II

Table III. Half-Lives ($t_{1/2}$) for the Disappearance of **4a** from Various Media

Medium	$t_{1/2}$ (min)	r^a
pH 7.4 phosphate buffer	7.7	−0.994
Rat 20% brain	11.0	−0.994
Rat 20% liver	8.5	−0.927
Rat 50% blood	30.2	−0.993

[a] r = correlation coefficient.

tization of a drug should substantially reduce its pharmacological/toxicological potential. The transport forms, especially the depoted pyridinium salt, should be inert. To examine this point, the ability of THA and **3a** to inhibit acetylcholinesterase *in vitro* was investigated using the Ellman method (42). As shown in Table IV, the IC_{50} for THA was 0.072 µ*M*, while the IC_{50} for **3a** was almost 100-fold higher (65 µ*M*) under equivalent conditions. These data are consistent with deactivation of the drug while it is being transported and stored.

In Vivo Distribution Study

The acute toxicity of THA and **4a** was examined in Sprague-Dawley rats. The maximum tolerated acute dose was found to be 30 mg/kg for **4a** and 2.8 kg for THA. A tissue distribution study was then performed. Rats were administered with the maximum tolerated doses for each compound as previously determined. The results are summarized in Figs. 1 and 2. When THA was administered to animals, peak levels were reached in the brain at 30 min (1.8 µg/g), after which the concentration rapidly decreased (5.3-fold in 2 hr and 346-fold in 6 hr); the first order half-life was 0.77 hr. The disappearance of THA from blood was rapid (half-life, 0.48 hr). Administration of **4a** gave a different distribution. High levels of the corresponding quaternary salt metabolite could be detected in the brain for a prolonged period. A concentration of 6.72 µg/g of the quaternary salt was rapidly reached (15 min). The level slightly increased (~7.9 µg/g after 30 min and 1 hr) and then slowly decreased; after 24 hr the level was 1.59 µg/g. The half-life of the quaternary salt in the brain was 10.7 hr. The quaternary salt was not detected in the blood (although an unidentified metabolite was present for a short while). It is obvious that **4a** was rapidly delivered to the CNS, followed by oxidation, and "locked-in" for a prolonged period. The stored quaternary salt was associated with a slow release of THA, which ranged from 3 to 15 ng/g in the brain. The brain level of THA was approximately the same after 24 hr, indicating the sustained hydrolysis of the metabolic precursor. When THA alone was administered, it was not detected in the brain 24 hr postinjection due to its bidirectional crossing of the BBB, equilibration with blood, and elimination.

Table IV. Effect of THA and **3a** on the Cholinesterase Activity of Purified Acetylcholinesterase[a]

Compound	Drug concentration (µ*M*)	Percentage activity (relative to control)
THA	2.93	2.21
	0.293	18.73
	0.0585	56.00
	0.0293	70.70
	0.0146	94.52
3a	888	6.24
	178	26.13
	35.5	68.4
	17.8	76.85
	8.88	93.85

[a] E.C. 3.1.1.7. Type III obtained from the electric eel.

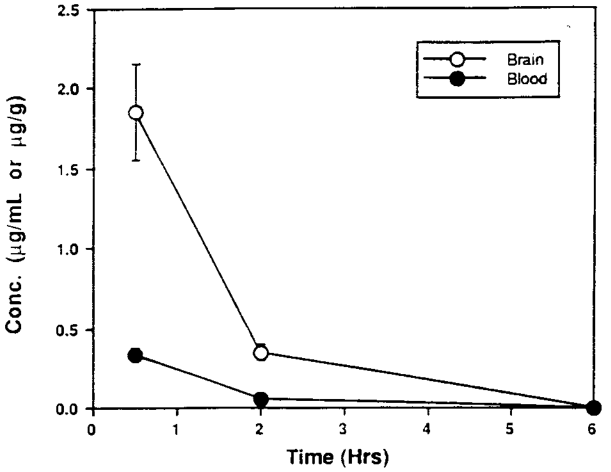


Fig. 1. THA concentration in brain and blood after administration of a 2.8 mg/kg i.v. dose of THA to rats.

In summary, one of the CDSs synthesized by THA was investigated. The derivative was sufficiently lipophilic to allow for rapid BBB transit and was sufficiently labile oxidatively to ensure rapid *in vivo* conversion of the dihydronicotinamide to the pyridinium salt. In addition, the depot form of the CDS, i.e., the quaternary salt, was not cholinomimetic in and of itself. Administration of the THA-CDS to rats resulted in prolonged levels at the corresponding salt in the CNS with rapid peripheral elimination. The lock-in salt slowly hydrolyzed producing THA in low but sustained lev-

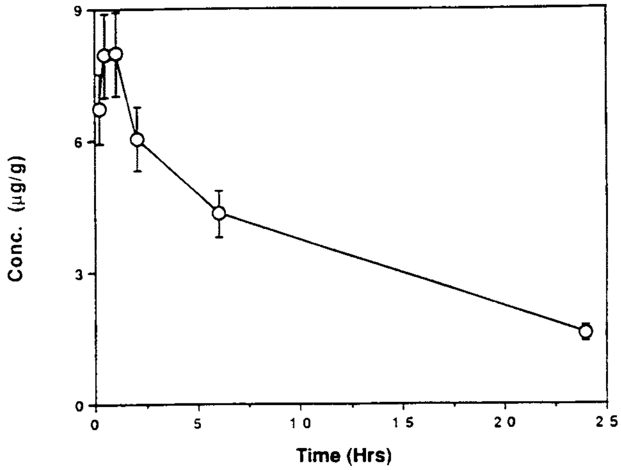


Fig. 2. THA-Q^+ (**4a**) concentration in brain after administration of a 30 mg/kg i.v. dose of **5a**.

els. The pharmacological significance of the release has not been assessed but similar CDSs for amino and diaminopyridine do appear to exert significant effects in various cholinergically associated deficit memory models (43).

ACKNOWLEDGMENTS

The authors would like to thank Dr. L. Prokai for mass spectrum determinations and Cindy Flowers for her help in preparing the manuscript. This work was supported by a grant from Pfizer, Inc., and by the NIH (GM 26167).

REFERENCES

1. W. Armstrong. *Recent Trends in Research on Alzheimers Disease,* PJB, Surrey, UK, 1968.
2. R. N. Katzman. *N. Engl. J. Med.* 314:964–973 (1986).
3. J. L. Cummings and D. E. Benson. In *Dementia: A Clinical Approach,* Butterworth, Boston, 1983, pp. 36–37.
4. R. T. Bartus, B. Beer, and A. S. Lippa. *Science* 217:408–417 (1982).
5. R. C. Mohs, F. C. S. Breitner, F. M. Silverman, and K. L. Davis. *Arch. Gen. Psychiat.* 44:405–408 (1987).
6. R. E. Becker and E. Giacobini. *Drug Dev. Res.* 12:163–195 (1988).
7. F. W. Hershenson and W. H. Moos. *J. Med. Chem.* 29:1125 (1986).
8. D. L. Price. *Annu. Rev. Neurosci.* 9:489 (1986).
9. M. D. Kopelman. *Q. J. Exp. Psychol.* 38A:535 (1986).
10. D. A. Drachman and J. Leavin. *Arch. Neurol.* 30:113–121 (1974).
11. P. F. Whitehouse, D. L. Price, R. G. Strubble, A. W. Clark, J. T. Coyle, and M. R. DeLong. *Science* 215:1237–1239 (1982).
12. N. R. Sims, D. L. Bowen, S. F. Allen, C. C. T. Smith, D. Neary, D. F. Thomas, and A. N. Davidson. *J. Neurochem.* 40:503–509 (1983).
13. C. A. Johns, V. Haroutunian, B. S. Greenwald, R. C. Mohs, B. M. Dais, P. Kanof, T. B. Horvath, and K. L. Davis. *Drug Dev. Res.* 5:77–96 (1985).
14. R. J. Wurtmann and J. D. Fernstrom. *Biochem. Pharmacol.* 25:1691–1696 (1976).
15. R. E. Harbaugh, D. W. Roberts, D. W. Coombs, R. L. Saunders, and T. M. Reedes. *Neurosurgery* 15:514–518 (1984).
16. K. A. Sherman, V. Kumar, J. W. Ashford, J. M. Murphy, R. J. Elble, R. Smith, and E. Giacobini. In R. Strong (ed.), *Central Nervous System Disorders of Aging: Strategies for Intervention,* Raven Press, New York, 1987, Vol. 33, pp. 71–90.
17. W. K. Summers, L. V. Majovaki, G. M. Marsh, K. Tachiki, and A. Kling. *N. Engl. J. Med.* 315:1241 (1986).
18. J. J. Marx. *Science* 238:1041 (1987).
19. G. M. Sgutske, F. A. Pierrat, M. L. Cornfeld, and M. R. Szewczak. *J. Med. Chem.* 31:1279–1282 (1988).
20. N. Bodor and H. Farag. *J. Med. Chem.* 26, 518–534 (1983).
21. W. Anderson, J. Simpkins, P. Woodard, D. Winwood, W. Stern, and N. Bodor. *Psychopharmacology* 97:157–163 (1987).
22. N. Bodor, T. Nakamura, and M. Brewster. *Drug Design Del.* 1:51–64 (1986).
23. N. Bodor, J. McCornack, and M. Brewster. *Int. J. Pharm.* 35:47–59 (1987).
24. M. Brewster, K. Estes, and N. Bodor. *Pharm. Res.* 3:278–285 (1984).
25. N. Bodor and H. Farag. *J. Pharm. Sci.* 73:385–389 (1984).
26. W. Anderson, J. Simpkins, M. Brewster, and N. Bodor. *Neuroendocrinology* 50:9–16 (1989).
27. E. Pop, E. Shek, T. Murakami, and N. Bodor. *J. Pharm. Sci.* 78:609–616 (1989).
28. K. Raghavan, E. Shek, and N. Bodor. *Anticancer Drug Design* 2:25–56 (1987).
29. N. Bodor, V. Venkatraghavan, D. Winwood, K. Estes, and M. Brewster. *Int. J. Pharm.* 53:195–208 (1989).
30. E. Pop, W.-M. Wu, E. Shek, and N. Bodor. *J. Med. Chem.* 32:1174–1781 (1989).
31. E. Pop, W.-M. Wu, and N. Bodor. *J. Med. Chem.* 32:1789–1796 (1989).
32. E. Pop, W.-M. Wu, E. Shek, and N. Bodor. *Drug Design. Del.* 5:93–115 (1989).
33. L. Prokai, B. H. Hsu, H. Farag, and N. Bodor. *Anal. Chem.* 61:1723–1728 (1989).
34. M. F. Powell, F. C. Wu, and T. C. Bruice. *J. Am. Chem. Soc.* 106:3850–3856 (1984).
35. E. Pop, M. E. Brewster, J. J. Kaminski, and N. Bodor. *Int. J. Quant. Chem.,* 35:315–324 (1989).
36. V. Petrow. *J. Chem. Soc.* 1947:634–637 (1947).
37. U. Eisner and J. Kutham. *Chem. Rev.* 72:1–42 (1972).
38. M. E. Brewster, A. Simay, K. Czako, D. Winwood, H. Farag, and N. Bodor. *J. Org. Chem.* 54:3721–3726 (1989).
39. C. Hansch and T. Fujita. *J. Am. Chem. Soc.* 86:1616–1626 (1964).
40. T. Fujita, J. Iwasa, and C. Hansch. *J. Am. Chem. Soc.* 86:5175–5180 (1964).
41. E. C. Bate-Smith and R. G. Westall. *Biochem. Biophys. Acta* 4:427–440 (1950).
42. G. L. Ellman, K. D. Courtney, V. Andres, Jr., and R. M. Featherstone. *Biochem. Pharmacol.* 7:88–95 (1961).
43. R. A. Allen, R. C. Effland, and J. T. Klein. U.S. Patent 4,578,394 (Hoechst-Roussel, Pharm.) (1986).