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Rational design of a drug for Alzheimer's disease with cholinesterase inhibitory and neuroprotective activity

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ABSTRACT

The rate and duration of inhibition of recombinant human acetylcholinesterase (AChE) and human butyrylcholinesterase (BuChE) by nine *N*-methyl,*N*-alkyl derivatives of (R)–3-prop-2-ynylamino-indan, designed as potential treatment of Alzheimer's disease, was obtained from measurement of the carbamylation k_i and decarbamylation k_3 rate constants. This also provided information about the rate of formation of the leaving group, 6-OH-(R)–3-prop-2-ynylamino-indan, designed as an MAO-B inhibitor with neuroprotective activity. The *N*-dimethyl derivative had the highest k_i of the alkyl derivatives. Substitution of one *N*-methyl by *N*-ethyl resulted in a 14-fold decrease in k_i and 28-fold decrease in k_3. A progressive increase in k_i occurred as the length of the alkyl chain progressed from propyl to *n*-hexyl and *cyclo*-hexyl, with relatively little or no increase in k_3. Higher k_i values than that of the dimethyl analogue were obtained with the *N*-aryl substitutes, *N*-phenyl and *N*-methoxy-phenyl. Six of the compounds had much higher k_i values for BuChE than AChE, but the *N*-*cyclo*-hexyl and *N*-methoxy-phenyl compounds were inactive. However, an inverse relation was found between k_i and the degree of brain AChE inhibition *ex vivo* after parenteral administration of the compounds in rats. This could have resulted from more rapid hydrolysis of the compounds with high k_i values by esterases in blood and liver. Only the *N*-ethyl and *N*-propyl derivatives showed AChE and BuChE inhibitory activity *in vivo* of a suitably slow onset and long duration, together with MAO-B inhibition.

1. Introduction

A progressive reduction in cholinergic transmission in the cortex and hippocampus contributes to the deficits in memory and cognitive function in Alzheimer's disease (AD) [1,2]. This observation led to the introduction of the acetylcholinesterase (AChE) inhibitors to prolong the duration of action of acetylcholine (ACh) and provide symptomatic treatment in this disorder [3,4]. AChE exists in multiple molecular forms that can be distinguished by their subunit associations [5]. In the mammalian brain most of the AChE is present in the membrane bound G4 form, but its levels decline as the neurons degenerate. Butyrylcholinesterase (BuChE) is expressed in glia and in selected areas of the central and peripheral nervous systems. It is capable of hydrolysing ACh, and its levels do not decline, or may even increase in AD [6]. Furthermore, selective BuChE inhibitors can maintain cholinergic transmission in AChE knockout mice and in rats with cholinergic lesions in which AChE levels are low [7,8]. Therefore drugs like rivastigmine that inhibit both AChE and BuChE may be preferable to selective AChE inhibitors, like donepezil, for the symptomatic treatment of AD [9].

The presence of oxidative–nitrative stress through the production of reactive oxygen species (ROS) and nitric oxide is more likely to occur in the aging brain because of a decline in antioxidant defence mechanisms. Oxidative stress occurs in the early stages of AD [10,11] and can readily be detected in the form of peroxinitrite [12]. ROS can attack proteins and membrane lipids causing progressive neurodegenera-

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tion. Therefore drugs that can either reduce the formation of ROS and/or protect cells from their damaging effects may decrease neurodegeneration and slow its progression. Effective therapy for AD is thus more likely to be achieved by drugs that incorporate both antioxidant and cholinesterase (ChE) inhibitory activity within the same molecule.

The monoamine oxidase-B (MAO-B) inhibitors, selegiline and rasagiline reduce the formation of H_2O_2 in the brain and prevent the effects of oxidative stress in a variety of models both *in vitro* and *in vivo* [13]. We therefore prepared a new series of compounds in which an *N*-methyl,*N*-alkyl carbamate moiety was incorporated in the 6-position of rasagiline to confer ChE-inhibitory activity [14]. Although this resulted in an almost complete loss of MAO-B inhibitory activity (IC_{50} > 500 μM), it was substantially retained in 6-OH rasagiline (IC_{50} 0.2 μM), the molecule that is formed after hydrolysis of the carbamate moiety by ChE (leaving group).

In designing a drug for the treatment of AD with both ChE-inhibitory and neuroprotective activity it is insufficient to base the selection on measurement of IC_{50} (concentration needed to inhibit ChE by 50%) at a single time point, as performed in the vast majority of studies [14–19]. This does not provide information about the rate of enzyme inhibition or its duration and thus potentially, the onset and length of drug action. These can be assessed from measurement of the rate of carbamylation, which governs both the onset of ChE inhibition and the formation of 6-OH-rasagiline, and of decarbamylation, respectively. A rapid onset of action is undesirable in AChE inhibitors, as it increases considerably the incidence of nausea and vomiting due to inhibition of AChE in the brainstem [20].

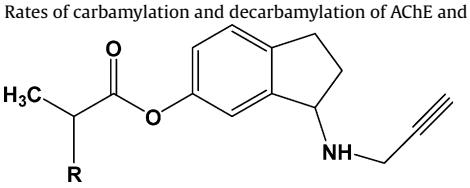
In the current study, we performed a kinetic analysis on the influence of the second substituent in nine *N*-methyl *N*-alkyl derivatives of (R)–3-prop-2-ynylamino-indan on the rates of carbamylation and decarbamylation of AChE and BuChE. The kinetic data were compared to those obtained with rivastigmine, an *N*-methyl-*N*-ethyl carbamate currently used in the treatment of AD. Rivastigmine is known to reach the brain fairly quickly and cause a relatively high incidence of adverse effects like nausea and vomiting [20]. These can be significantly reduced by slowing its rate of access to brain AChE by administering the drug via a transdermal patch [21].

2. Materials and methods

Recombinant human AChE and human BuChE, found by sedimentation on sucrose gradient to consist mainly of the tetrameric G4 form, were purchased from Sigma–Aldrich Israel. The enzymes were dissolved in phosphate buffer pH 8 containing 0.01% NaN_3 and 1mM EDTA to give a final concentration of 20 U/ml and stored at −20 °C until use. Acetylthiocholine iodide (ATC), butyrylthiocholine iodide (BTC), bovine serum albumin (BSA), and 5,5′-dithiobisnitrobenzoic acid (DTNB) were purchased from Sigma–Aldrich Israel.

Enzymic activity was determined at 37 °C by the method of Ellman et al. [22] using ATC as a substrate for rhAChE and BTC as a substrate for hBuChE on a multiscan microplate reader (Labsystems) containing 96 wells. Each well contained rhAChE 0.02 U/ml or BuChE 0.025 U/ml, DTNB 0.25 mM, ATC or BTC 1 mM, phosphate buffer pH 8.0 (0.1 M) and BSA (0.05%), NaN_3 (0.01%) and 1 mM EDTA, in a total volume of 0.2 ml. Several of the inhibitors tested in this study were R-enantiomers as most of the MAO-B inhibitory activity resides in this form, and the remainder were racemic mixtures. Unlike rivastigmine, no significant difference was found in the kinetics of inhibition of AChE by the S and R isomers [23]. Their structures are shown in Table 1. All doses and concentrations of drugs are in terms of their hydrochloride salt with the exception of the *N*-ethyl compound which is a hemitartrate.

Table 1
Rates of carbamylation and decarbamylation of AChE and BuChE by novel carbamates



R	AChE			BuChE	
	k_i ($\times 10^3$ M^{-1} min^{-1})	Relative activity[a]	k_3 ($\times 10^3$ min^{-1})	k_i ($\times 10^3$ M^{-1} min^{-1})	k_3 ($\times 10^3$ min^{-1})
Methyl	10.1 ± 1.0	222	8.4 ± 0.4	21.7 ± 0.4	6.3 ± 0.2
Ethyl	0.7 ± 0.1	15	0.30 ± 0.01	16.5 ± 0.1	2.4 ± 0.1
Propyl	1.4 ± 0.1	24	0.38 ± 0.02	8.3 ± 0.1	1.2 ± 0.2
Butyl	5.4 ± 0.4	119	0.40 ± 0.08	30.7 ± 0.6	0.8 ± 0.2
n-Hexyl	8.3 ± 0.3	183	0.31 ± 0.01	41.0 ± 1.1	1.2 ± 0.1
cyclo-Hexyl	8.9 ± 0.2	196	(<5% at 92 h)	Inactive[b]	Inactive[b]
Phenyl	25.6 ± 1.3	564	1.6 ± 0.04	4.1 ± 0.5	3.4 ± 0.5
Benzyl	3.9 ± 0.1	86	0.20 ± 0.01	50 ± 5	0.90 ± 0.02
Methoxy-phenyl	14.1 ± 0.6	311	0.60 ± 0.01	Inactive[b]	Inactive[b]
Rivastigmine	4.5 ± 0.1	100	0.31 ± 0.01	333 ± 22	2.3 ± 0.3

[a] Relative to that of rivastigmine.
[b] No activity at 400 μM.