Stereospecific Inhibition of Cholinesterases by Mefloquine Enantiomers

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Mefloquine enantiomers (+)-1, (−)-1 were found to be stereospecific in their inhibition of acetylcholinesterase and butyrylcholinesterase, (−)-1 being the more potent inhibitor in both cases. Similar observations were also made with respect to (−)-quinine and (+)-quinidine, which are configurational analogues of (+)-1 and (−)-1, respectively. A positive synclinical conformation for the “N-C-C-O” segment of mefloquine appeared to be necessary for good activity.

Keywords—mefloquine; enantiomers; stereospecific; anticholinesterase; quinine; quinidine

Introduction

Structure–activity relationship studies of inhibitors of acetylcholinesterase (AChE) have shown that these agents generally possess a positively charged nitrogen which is separated from an electro-negative oxygen by two or three carbon atoms, as in the N–C–C–O segment of acetylcholine. The positively charged nitrogen interacts with the anionic site of the enzyme, while the electro-negative oxygen interacts with an acid group via hydrogen bonding. It should be noted that as a chemical class, aminoethanols have the necessary N–C–C–O segment for anti-AChE activity. The nitrogen atom of aminoethanols (pKₐ 9.5) is protonated at pH 7.4, carrying a positive charge, while the hydroxyl oxygen is inherently electronegative.

Mefloquine [(±)-1] is one of the best known and most widely used aminoalcohol antimalarials discovered during the mid-1960s under an extensive research program sponsored by the US Army Medical Research and Development Command.¹ Synthetic mefloquine is a racemate, the erythro isomers of α-(2-piperidinyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol. Thus, mefloquine is structurally an aminoethanol, with chiral C9 and C10 (see Fig. 1). Since it has a pharmacophoric N–C–C–O segment (i.e. N11–C10–C9–O16, Fig. 2), mefloquine is expected to be an inhibitor of AChE, and the comparative inhibitory activities of its enantiomers, whose N–C–C–O segments are mirror images of one another,

![Fig. 1. Structural Formulae of (+)-1, (−)-1, 2 and 3](image-url)
would shed some light on the structural and conformational requirements of the enzyme receptor.

In the present study, the anti-AChE and anti-butrylcholinesterase (anti-BChE) activities of (+)-1 and (−)-1, as well as that of the racemate, were determined at pH 7.4 using a pH-stat method. The activities of (−)-quinine (2) and (+)-quinidine (3) were similarly studied.

**Experimental**

(−)-Quinine sulfate [(8S,9R)-6'-methoxy-cinchonan-9-ol sulfate] and (+)-quinidine [(8R,9S)-6'-methoxy-cinchonan-9-ol sulfate] were obtained from British Drug House and the racemic mefloquine hydrochloride [(±)-(R*,S*)-2,8-bis(trifluoromethyl)-α-(2-piperidinyl)-4-quinolinemethanol] was supplied by Roche Pharmaceuticals. The racemic mefloquine was resolved with (+)-3-bromo-8-camphorsulphonic acid in methanol according to the method of Carroll and Blackwell.2) (+)-1 and (−)-1 were obtained in 26% and 11% yields, respectively. The melting points and specific rotation of the resolved enantiomers were in agreement with reported values.3

A modification of the constant pH titration method of Stanely et al. was followed for the enzyme inhibition studies.3 At least two concentrations of each inhibitor were employed for the determination of inhibitory activity. All glassware used for the preparation and storage of solutions containing inhibitors was initially coated with dimethyldichlorosilane. (+)-1 and (−)-1, isolated as the free bases, were each dissolved in a minimum volume of 10% (v/v) 0.1 M HCl and made up to volume with double-distilled water, while solutions of the other inhibitors were prepared in double-distilled water. Enzyme activity was determined at pH 7.4 and 23 °C, using a Radiometer TTT2 autotitrator assembly. For the determination of AChE activity, a solution of electric eel AChE (EC 3.1.1.7, Sigma Type VI-S), containing 4 units/ml, was prepared in 0.002 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.01% (w/v) gelatin. The hydrolysis of the substrate acetylcholine iodide (Sigma Chemical Co.), over the concentration range of 0.8—1.5 mm, was determined in 20 ml of 0.1 M MgCl2 solution. The hydrolysis was initiated by addition of 0.4 units of the enzyme and the acetic acid liberated was titrated with 0.005 M NaOH over a period of 4 min. The reaction velocity (moles of alkali consumed per minute per unit of enzyme) was determined from the slope of the curve (ml of alkali versus time) between 2 and 4 min. The Michaelis constant of AChE was found to be constant at 2.38 × 10−4 M (S.E.M 0.15 × 10−4 M for 12 determinations). For the determination of BChE activity, horse serum BChE (EC 3.1.1.8, Sigma Type XI) containing 17.2 units/ml was similarly prepared in phosphate buffer. Hydrolysis of ACh iodide over the concentration range of 1.6—0.2 mm, by 1.72 units of the enzyme, was determined in 20 ml of 0.1 M MgCl2. The reaction velocity of each hydrolysis was determined by titration with 0.01 M NaOH over 4 min as in the case of AChE. The Michaelis constant for BChE was found to be 1.05 × 10−3 M (S.E.M 0.52 × 10−3 M for 12 determinations). The reaction velocities were determined in the presence and absence of inhibitor for each enzyme. The results were analyzed by means of ROSFIT,4 a computer program written for the discrimination between rival inhibitory models and for estimation of kinetic parameters. The inhibitory constants of the compounds are listed in Table I.

**Results and Discussion**

Mefloquine [(±)-1] and its enantiomers, (+)-1 and (−)-1, were found to be non-competitive inhibitors of AChE and BChE in the present study. Neostigmine, an established anti-AChE agent, exhibited competitive kinetics under similar experimental conditions. Table

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i \times 10^4$ (M$^{-1}$)</th>
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<tbody>
<tr>
<td></td>
<td>AChE</td>
</tr>
<tr>
<td>(+)-1</td>
<td>0.445 (± 0.0049)</td>
</tr>
<tr>
<td>(+)-1</td>
<td>1.03 (± 0.025)</td>
</tr>
<tr>
<td>(−)-1</td>
<td>0.288 (± 0.0045)</td>
</tr>
<tr>
<td>2</td>
<td>8.40 (± 0.14)</td>
</tr>
<tr>
<td>3</td>
<td>5.72 (± 0.072)</td>
</tr>
</tbody>
</table>

a) Values in parentheses indicate standard error of the mean (S.E.M.) for 20 observations.
I gives the $K_i$ values of mefloquine and its enantiomers against AChE and BChE, computed from the ROSFIT model for classical non-competitive inhibition. As expected, one of the enantiomers, (-)-1, was found to be much more active than its optical antipode, (+)-1, as an inhibitor of AChE. The same was also true with respect to inhibition of BChE. The activity of racemic mefloquine was intermediate between those of the enantiomers.

Carroll and Blackwell had previously assigned the absolute configurations of (+)-1 and (-)-1 as $C9\ (R)\ C10\ (S)$ and $C9\ (S)\ C10\ (R)$ respectively. This was done by comparing the circular dichroism spectra of (+)-1 and (-)-1 with those of (-)-quinine (2) and (+)-quinidine (3), the absolute configurations of which have been reported. It is clear from their study that (+)-1 and (-)-1 are configurational analogues of 2 and 3, respectively. It would be interesting to find out whether 2 and 3 show similar stereospecificity in their inhibitions of cholinesterases. This was indeed found to be the case in the present study. (+)-Quinidine was more active than (-)-quinine against both AChE and BChE (Table I). Thus, there is in this case a correlation between the absolute configurations and anti-cholinesterase activities among the stereoisomers.

It appears from Table I that the difference in anti-ChE activities between 2 and 3 is more pronounced with respect to BChE than AChE. This may be due to the fact that the steric requirement of the AChE anionic site is more stringent than that of BChE. The nitrogen of the N–C–C–O segment of quinine and quinidine is located at the bridge head of the bulky quinuclidine ring. Attachment of the N–C–C–O segment to the enzyme receptor would not be easy, and the situation would be more difficult with AChE. Thus, the $K_i$ values of 2 and 3 against AChE are comparatively high, but nevertheless 3 is still a better inhibitor.

Conformational study using Dreiding models of the mefloquine enantiomers has shown that when C9 is equatorial to the piperidine ring, the conformer with the least steric interaction is the one in which the quinoline ring and the piperidinyl nitrogen are anti to each other, i.e. $\tau C4-C9-C10-N11 = 180\ ^\circ C$ (Fig. 2). The stability of this conformer may also be enhanced by a possible, though weak, internal hydrogen bonding between the protonated piperidinyl nitrogen (at pH 7.4) and the hydroxyl group. It is reasonable to expect this stable conformer to play a major role in the interaction of mefloquine with the cholinesterase receptors. One should mention that in a parallel conformational study of an analogous group of aminoalcohols—the $\alpha$-piperidinyl-3,6-bis-(trifluoromethyl)-9-phenanthrenemethanols—Loew and Sahakian have similarly concluded that the most stable conformers are those in which the bulky phenanthrene ring and the piperidinyl nitrogen are anti to each other. There is no reason to suspect that (+)-1 and (-)-1 would behave otherwise.

Thus, with $\tau C4-C9-C10-N11$ of the mefloquine enantiomers at $180\ ^\circ C$, the corresponding N11–C10–C9–O16 torsion angle is either $+60\ ^\circ$ or $-60\ ^\circ$, depending on the absolute configuration of the enantiomers. Taking into consideration the absolute configurations of (+)-1 and (-)-1 as assigned by Carroll and Blackwell, the N11–C10–C9–O16 torsion angle of (+)-1 is $-60\ ^\circ$ and that of (-)-1 is $+60\ ^\circ$. As the N–C–C–O segment of mefloquine has been identified as the pharmacophoric moiety for anti-ChE activity, one may conclude that a positive synclinal conformation, as found in (-)-1, is essential for good anti-ChE activity.
References