Poster Session 1 – Pharmacology

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Studies on the antiprotease potential of tacrine and related compounds

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Drug strategies aimed at slowing the progress of Alzheimer's disease have focused on inhibition of two enzyme systems: cholinesterases, in an attempt to restore cholinergic function, and proteases, in order to prevent abnormal protein processing pathways that lead to the observable pathological changes in brain tissue (Sharma *et al* 1997). Tacrine is an anticholinesterase agent with an established role in the management of Alzheimer's patients (Shumock 1998). Following preliminary observation of the ability of tacrine to inhibit calcium dependant protease activity (Qayum *et al* 1994) we have compared the ability of some related compounds to inhibit these enzymes in model in-vitro systems, and determined possible mechanisms of interaction with protease enzymes.

Acetylcholinesterase activity was determined by measuring the reduction in absorbance of 3-nitrophenol at 450 nm. Electric eel acetylcholinesterase (Sigma) was used at a starting activity of 10 U mL^{-1}, where 1 U is equivalent to liberation of 1 μmol of acetic acid from acetylcholine at pH 8.0 and 37°C. The assay mix contained 50 mM actetylcholine chloride, 50 mM 3 nitrophenol and 0.1 M phosphate buffer (pH 8.0) in a total volume of 0.25 mL. Maximum linear rate during a 20-min interval was taken as a reliable measure of enzyme activity, and assessed against a blank containing test drug and appropriate vehicle where appropriate.

The activity of calcium-dependent neutral protease was determined turbidimetrically using *N*,*N*-dimethylated casein as substrate. Rabbit muscle calpain (Sigma) was used at a starting activity of 0.1 U mL^{-1}. The assay mix contained 1 mM dithiothreitol, 5 mM calcium chloride and 4 mg mL^{-1} substrate in Tris-Cl buffer (50 mM, pH 7.5), in a total volume of 0.25 mL and at 37°C. Turbidity increase was measured over a 4-h period as increase in absorbance at 550 nm. This was compared with appropriate blank incubations. In kinetic studies, the calcium concentration was varied to a maximum of 5 mM.

In both systems, full activity ranges were evaluated in at least three separate experiments. Tacrine and related compounds 9-aminoacridine and 4-aminopyridine were found to be dual inhibitors at broadly comparable concentrations in the test systems used (Table 1). Kinetic analysis of the inhibition of protease by tacrine indicated that the compound was competitive with respect to calcium binding.

Table 1 Dual inhibition of acetylcholinesterase and calpain by test compounds

Drug	IC50 (M)	
	Acetylcholinesterase	Calpain
Tacrine	5.2×10^{-5} (± 5.7)	7.0×10^{-5} (± 3.5)
9-Aminoacridine	8.0×10^{-6} (± 3.0)	2.0×10^{-5} (± 1.3)
4-Aminopyridine	1.0×10^{-4} (± 6.2)	3.2×10^{-5} (± 4.8)

(±s.e.m.)

It is possible that antiprotease and anticholinesterase activity are linked in other chemical series, providing potentially greater benefits in the treatment of Alzheimer's disease than either strategy alone.

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