Assessing the Reversibility of Acetylcholinesterase (AChE) Inhibitors

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Abstract
Inhibition of Acetylcholinesterase (AChE) may cause severe toxicities potentially leading to death; but the severity of the side effects is partly depending on the reversibility of the inhibition. AChE is one of the consensus targets against which new drug candidate compounds are commonly profiled in vitro assay panels (such as our renowned Eurofins Cerep SafetyScrees Panel), but these assays do not distinguish between reversible and irreversible inhibitors.

We have now developed a new in vitro assay for assessing the reversibility of AChE inhibitors. Briefly, after pre-incubation with the test compound, the enzyme is dialyzed for different periods of time to remove unbound inhibitor, and then the AChE activity is measured in the usual colorimetric assay. The stability of the enzyme and test compounds is tested in parallel for control. We have validated our new assay with different reference compounds, and demonstrate that our assay clearly distinguishes between reversible (e.g. Tacrine) vs. irreversible (e.g. DIFP) AChE inhibitors.

Finally, using our new assay, we have tested several sets of compounds from AstraZeneca looking at the reversibility of their AChE inhibition. Most of these compounds displayed fast reversible kinetics but some showed differential kinetics where although reversible the rate was far slower. The results were very helpful in allowing an enhanced risk assessment and allowing for better prioritization of compounds from a safety perspective.

We propose that any compound detected as an AChE inhibitor in a safety pharmacology profile should be systematically tested in a follow-up mechanism-of-action study to assess the reversibility of the inhibition. Our newly developed AChE inhibition reversibility assay is now available as a new standard assay at Eurofins Discovery (Eurofins Cerep ref. 4842).

Methods
AChE enzymatic activity was measured in a standard colorimetric assay (Eurofins Cerep ref. 363). To assess the reversibility of AChE inhibitors, the enzyme was pre-incubated (30 minutes) with the test compounds (at - IC50), then dialyzed (at 4°C) for different periods of time (0 / 4 / 24 / 48 h) to remove unbound inhibitor, and then the AChE activity was measured using the standard assay. The stability of the enzyme and the test compounds was systematically tested in parallel for control.

Assay Development: Stability of the Enzyme Over Time

Figure 1. Assay development: Stability of the enzyme over time. The AChE enzyme was incubated for 0 / 4 / 24 / 48 h at 4°C (similar to the dialysis conditions in the reversibility assay), then the enzyme activity was assessed. A. AChE activity in absence of inhibitor (n=4 +/- sd). B. AChE activity in presence of the reference inhibitor Neostigmine (n=2 +/- sd). The AChE enzyme is stable and fully functional for at least 48 h at 4°C.

Test of the AstraZeneca Compounds

• Several compounds from AstraZeneca were tested in this assay, providing results highly useful for an enhanced safety risk assessment where fully reversible compounds would be preferentially advanced over those with slow off rate.
• Compounds detected as AChE inhibitors in a safety pharmacology profile could be systematically tested in a follow-up mechanism-of-action study to assess the reversibility of the inhibition. The assay is now available at Eurofins Discovery (Eurofins Cerep ref. 4842).

Summary
• We have successfully developed an in vitro assay for assessing the reversibility of AChE inhibitors.
• The assay clearly distinguishes between reversible, slowly reversible and irreversible AChE inhibitors such as Tacrine, Neostigmine and DIFP, respectively.
• Several compounds from AstraZeneca were tested in this assay, providing results highly useful for an enhanced safety risk assessment where fully reversible compounds would be preferentially advanced over those with slow off rate.
• Compounds detected as AChE inhibitors in a safety pharmacology profile could be systematically tested in a follow-up mechanism-of-action study to assess the reversibility of the inhibition. The assay is now available at Eurofins Discovery (Eurofins Cerep ref. 4842).

References