New York University and Aria Neurosciences investigators have confirmed with 2-([pyridine-2-ylmethyl]-amino)-phenol (2-PMAP) that inhibition of amyloid-β precursor protein (APP) synthesis offers an alternative to and possibly a significant advance over existing approaches to Alzheimer’s disease (AD). As the repeated clinical trial failures in AD warn, along with all other AD investigators they now face daunting tasks ahead. Methods and knowledge of AD pathological mechanisms may not be available to successfully time 2-PMAP interventions early enough in the course of AD to prevent possible amyloid β-induced irreversible pathologies such as phosphorylated-tau protein. It may not be possible to meet US Food and Drug Administration clinical efficacy requirements during preclinical stages decades earlier when clinical pathology cannot be reversed but only prevented possibly years later. Methods used in AD drug development currently may be a barrier of equal importance to the identification of candidate drugs.

Methodological problems are seen in the report from Asuni et al. who attempted to compare 2-PMAP to phenserine, another APP synthesis inhibitor. They are to be commended for seeking comparisons to an existing drug in the same class, rather than a less informative comparison to placebo. Unfortunately, their choice of methodologies invalidates the comparisons they offer. We bring these technicalities to readers’ attention to illustrate how methodological perils can mislead investigators, confuse readers, and confound AD drug developments.
By using transfected Chinese hamster ovarian (CHO) clones, not using neuronal cell cultures with intact APP synthesis regulatory elements,[4] and risking variable drug bioavailability, the authors reported a misleading minimal effective concentration for phenserine of 5 to 25μM.[1] Unlike outcomes in neuronal cells,[4, 5] we found phenserine relatively inactive using CHO clones (unpublished data). Phenserine is also highly lipophilic (clog P n-octanol/water partition value = 2.22 (vs. 0.925 for 2-PAM) (i.e., a 160-fold preference for the lipid vs. aqueous phase for phenserine vs. 8-fold for 2-PAM)). We found it necessary to use the tartrate salt in preclinical/clinical studies to provide reliable aqueous bioavailability.[4] The use by Asuni and colleagues[1] of free base phenserine risks functional concentrations far lower than they reported.

Readers not familiar in depth with human clinical pharmacology could easily miss that Asuni and colleagues’ reported micromolar phenserine concentrations are unachievable in human brain and therefore in conflict with published data. We expect Asuni and colleagues would have recognized and explained this discrepancy for readers, because they cite Lahiri et al.[4] in which we reported the nonchiral phenserine extracellular and intracellular median inhibition concentration (IC50) values for APP synthesis in neurons (0.64–1.0 and 1.14–1.5μM, respectively) (this data derived from a concentration-dependent study to define the IC50[4], albeit higher phenserine doses have been used in earlier studies). The literature also documents for Asuni and colleagues the relevant rapid chiral metabolism of (−)-phenserine,[6] the cellular IC50 of 100nM for the resulting active N1,N8-bisnor-metabolite, [5] and, of most importance, the lowering of cerebrospinal fluid APP and key AD markers in humans with mild cognitive impairment (i.e., clinical studies rather than cell culture ones). [7] Each of these is inconsistent with the implications to be drawn from Asuni and colleagues’ reported values, which require explanation to avoid misleading readers.

The presence of these inconsistencies in Asuni and colleagues’ article should warn readers of the importance of methodologies. Methodologies affect study outcomes.[2] Investigators bear responsibility for disconfirming results using the original investigators’ methods before claiming and using disconfirming data in an active control comparison. Publishing misleading characterizations of a drug will only harden the resistance of pharmaceutical firms to provide their compounds to academic investigators, thus undermining the more informative comparisons of new drugs to existing drugs rather than to placebo. Journals are uniquely positioned to inform nonspecialists of how methodologies impact study outcomes.

As a major advance in AD pharmacology, 2-PMAP may have interesting theoretical importance as a selective APP synthesis inhibitor. Phenserine has an apparent different mechanistic target on mRNA regulatory elements [5] not present in transfected CHO cell lines and neuroprotective, neurogenic, and anti-inflammatory activities that may or may not prove useful in addressing the complex AD neuropathologies. Further comparisons of the two drugs should help clarify the utility of specific anti-APP and combined APP, neuroprotective, anti-inflammatory treatments in AD.
References


