

New Therapeutic Strategies for the Treatment of Alzheimer's Disease

Linda J. Van Eldik, Jeffrey M. Craft, Wenhui Hu, Hantamalala Ralay-Ranaivo,
and D. Martin Watterson

Center for Drug Discovery and Chemical Biology, Northwestern University, Chicago IL USA

Alzheimer's disease (AD) has an unmet medical need for therapies that target disease progression. Chronically activated glia (astrocytes and microglia) contribute to neuronal dysfunction through generation of proinflammatory cytokines and oxidative stress molecules. Neuronal dysfunction, in turn, can lead to further glial activation and propagation of a localized, detrimental neuroinflammation cycle. The neuroinflammation hypothesis posits that attenuation of this detrimental cycle is a potential point of intervention in AD progression. We recently validated this hypothesis using an integrative chemical biology approach that employed bioavailable pyridazine-based inhibitors and a new mouse model of human amyloid-beta1-42 (A β)-induced hippocampal injury [1-4]. We developed compounds that could selectively suppress glial neuroinflammatory responses with a resultant attenuation of hippocampal neuronal dysfunction, neuronal loss, and spatial learning deficits [2,3,4]. The suppression of neuroinflammation by these bioavailable compounds was accomplished without suppression of the peripheral immune system over the effective dose range, and without inducing tissue toxicities or prolongation of QT interval. These results provided an initial validation for targeting excessive glial activation as a drug discovery approach, and indicated that there are critical signaling pathways in the central nervous system innate immune system that are quantitatively distinct from those that converge on the same end points, e.g., proinflammatory cytokine production, in the peripheral immune system.

These novel integrative chemical biology results demand that attempts be made to translate these basic science findings into a search for safe and effective, AD-relevant lead compounds with oral bioavailability. AD therapeutics must be comparatively safe due to the anticipated chronic use and age of the patient population, and oral bioavailability is desired for patient compliance and ease of administration. Therefore, we sought to extend our initial target validation results [1-3] toward lead compound development by chemical diversification of the privileged pyridazine core fragment. A focused library of pyridazine compounds was synthesized and taken through our hierarchical biological screening process involving a) cell-based screening for concentration-dependent and selective inhibition of glia inflammation responses, and b) *in vivo* assays using mouse models of A β -induced neuroinflammation, synaptic dysfunction and neuronal death, in order to discover compounds with *in vivo* anti-neuroinflammatory activity. An oral toxicological screen was also incorporated into the discovery process [4] by using a multi-day, escalating dose standard protocol, as well as tissue analysis after the end of a two week therapeutic dose regimen.

We report here that safe, effective, orally bioavailable lead compounds have been developed using an animal model of AD-related disease progression as a biological endpoint for efficacy. These compounds suppress production of neuroinflammatory cytokines and other biomarkers indicative of human A β -induced neuroinflammation, and protect against neuronal dysfunction in the hippocampus. These first generation experimental therapeutics are amenable to further medicinal chemistry refinement in future drug development with retention of good computed molecular properties. Current research is focused on medicinal chemistry refinement and further safety evaluations as a foundation for phase I clinical investigations.

- [1] Mirzoeva, Sawkar, Zasadzki, Guo, Velentza, Dunlap, Bourguignon, Ramstrom, Haiech, Van Eldik and Watterson (2002) Discovery of a 3-amino-6-phenyl-pyridazine derivative as a new synthetic anti-neuroinflammatory compound. *Journal of Medicinal Chemistry* 45: 563-566.
- [2] Craft, Watterson, Frautschy and Van Eldik LJ (2004) Aminopyridazines inhibit β -amyloid induced glial activation and neuronal damage *in vivo*. *Neurobiology of Aging* 25: 1283-1292.
- [3] Craft, Van Eldik, Zasadzki, Hu, Watterson (2004) Aminopyridazines attenuate hippocampus dependent behavioral deficits induced by human β -amyloid in a murine model of neuroinflammation. *Journal of Molecular Neuroscience* 24: 115-122.
- [4] Hu, Ranaivo, Craft, Van Eldik and Watterson (2005) Validation of the neuroinflammation cycle as a drug discovery target using integrative chemical biology and lead compound development with an Alzheimer's disease-related mouse model. *Current Alzheimer's Research*, in press.

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