A Toxidromic Approach for Chemical Medical Countermeasure Development

Selective Staining of Genetically Modified Mouse Strains as Part of an Initial Strain Characterization Study

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Acetylcholinesterase serves a vital role in muscle contraction and cholinergic neural pathways by hydrolyzing the neurotransmitter acetylcholine and terminating synaptic stimulation. For this reason, organophosphorus (OP) nerve agents that are capable of covalently binding to the active site of this enzyme cause inhibition of the endogenous hydrolytic activity; without the breakdown of acetylcholine at the synapse, nerve signaling is not terminated, leading to a systemic cholinergic crisis and eventual death. Effective in vivo models that accurately predict human responses are critical for the study of the lethal effects of OP nerve agents and the evaluation of novel OP nerve agent countermeasures. Although mice are often utilized to study the effects of OP nerve agents, mice possess the endogenous bioscavenger serum carboxylesterase that sequesters some OP nerve agents away from the central nervous system, thereby offering some protection against intoxication. Since humans lack this endogenous bioscavenger, mice are an inaccurate model for human intoxication by OP nerve agents. Furthermore, recent studies have shown significant biochemical differences in human and mouse acetylcholinesterase interactions with small molecules, particularly potential reactivators of OP-inhibited enzyme. To better study OP nerve agent intoxication and the efficacy of potential nerve agent countermeasures, a mouse strain was recently created that incorporates two genetic modifications: the removal of functional serum carboxylesterase and the substitution of human acetylcholinesterase for mouse acetylcholinesterase. In this study, we sought to characterize the expression of human acetylcholinesterase in 10- to 13-week-old mice of four different genotypes: wild type (WT), serum carboxylesterase knock out (Es1 KO), human acetylcholinesterase knock in (AChE KI), and the knock in/knock out combination (KIKO). Staining the brain tissue of these animals with a fluorescein isothiocyanate-linked antibody specific for mouse acetylcholinesterase confirmed the absence of mouse acetylcholinesterase expression in the brains of the AChE KI and KIKO animals. The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division. This research was supported in part by an appointment to the Postgraduate Research Participation Program at the U.S. Army Medical Research Institute of Chemical Defense administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U. S. Army Medical Research and Materiel Command.