CAMO (COMPARING ANIMAL MODELS TO ORGANOIDS) - TESTING MEDICAL COUNTERMEASURES WITH MICROPHYSIOLOGICAL SYSTEMS AND COMPARING TO TRADITIONAL ANIMAL MODELS AND CLINICAL TRAILS

Characterization Of A Humanized Mouse Model Of Organophosphate Nerve Agent Poisoning And Detection Of Countermeasures Via Maldi-msi.

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Organophosphorus nerve agent (OPNA) poisoning leads to a cholinergic crisis resulting in difficulty breathing, seizures, and death due to the inhibition of acetylcholinesterase (AChE). This process can be mitigated with the use of reactivators which interact with the bound OPNA, releasing it from the enzyme active site. Many questions remain surrounding the treatment of OPNA intoxication, including if reactivators can cross the blood brain barrier. Studying the effects of OPNA poisoning and reactivators is hindered by two common issues: 1) small animal research models produce serum carboxylesterase (CaE) that acts as an endogenous bioscavenger of OPNAs, and 2) minor amino acid differences in AChE across species cause differential interactions with small molecules intended to restore native activity of the OPNA-inhibited enzyme. The KIKO (AChE Knock-In/CaE Knock-Out) mouse model incorporates two genetic modifications into a single animal that addresses these concerns. First, serum CaE, removed in KIKO mice, is known to directly contribute to increased resistance to toxicity by OPNA compounds as compared to humans and nonhuman primates. Additionally, CaE has been shown to affect the pharmacokinetic profile and efficacy of other pharmaceuticals. Second, the production of solely the human form of AChE in the KIKO mice presents a unique opportunity for this animal to act as a model for the study of compounds which interact directly with the enzyme. Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry Imaging (MSI) utilizes mass spectrometry on tissues while maintaining spatial resolution, unlike traditional mass spectrometry approaches. This method has been utilized to examine endogenous small molecules including neurotransmitters and metabolites, tryptic peptide, lipids, and glycans, all without modification to the tissue. We used the MALDI-MSI technique to elucidate the effects of altered AChE expression as a function of both age and sex in an effort to better characterize the humanized KIKO mouse model as compared to CaE KO and WT (C57Bl/6J) strains across pediatric, young adult, mature adult, and geriatric populations. We found no significant changes in AChE localization or abundance between strains, however there were several differences present between both sexes and age groups. We then sought to leverage this system to definitively answer the lingering question of whether standard medical countermeasures (MCMs) are able to cross the blood brain barrier. Counter to the commonly held standard that positively charged molecules cannot cross the blood brain barrier, we detected a small amount of reactivator within the brain of both exposed and unexposed animals. Our data also confirms the presence of increased acetylcholine in OPNA-exposed animals, regardless of treatment status. Further, we were able to simultaneously image as well as determine the location of both reactivator and acetylcholine in the brain. These efforts establish a technique that can be utilized in the characterization of a variety of drug candidates thought to be active in the central nervous system by allowing spatially relevant imaging of unmodified drug forms at molecular resolution.

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