Biomarkers of Organophosphate-Adducted Proteins

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This work seeks to identify adducted and altered protein biomarkers resulting from the covalent modification of proteins by organophosphate (OP) insecticides. Millions of pounds of OP insecticides are used each year leading to tens of thousands of reports to poison control centers each year, however, these reports only account for acute poisoning events and do not include low dose or chronic exposures. In addition to general exposure and food contamination, the safety of OPs is a large public health concern because OPs share chemical traits, structure and biological mechanism with chemical nerve gas agents. These concerns are elevated by an array of neurologic and non-neurologic sequelae that have been reported in connection with exposure to OPs. Despite a long history of use in the U.S. and worldwide, there are few tests for OP detection that correlate with exposure that show adequate sensitivity and specificity. To date, the blood cholinesterase test (BCT) has been conducted but is seriously limited as an enzyme activity-based assay to assess OP exposure. A detection method is needed that examines true biomarkers of exposure. More specifically, new tests are needed that are sensitive, selective, operate in real time and are based on reliable, well-characterized OP-biomarkers derived from exacting molecular events that correlate with cellular, tissue, organ or systemic toxicity.

One of the more distinctive features of OP mode of action is that the agent forms a covalent bond with its primary target, acetylcholinesterase (AChE). In doing so, the OP agent modifies the proteins and deposits a specific signature that correlates with a specific gain in molecular weight. To this end, most OP insecticides fall into two distinct classes of modifiers (dimethoxy- and diethoxy-OPs), which renders the identification more facile. Also, due to rapid advances in mass spectrometry, the exact location of this OP chemical modification can be analyzed and determined to reveal the identity of the amino acid residue that has been modified by the OP. Once the OP chemical structure and the location of the modification have been identified, the peptide region containing the OP-modified amino acid residue will be prepared and used as haptens to generate antibodies specific to this OP-adducted protein. These antibodies will be used to detect these protein adducts as mechanism-based biomarkers of OP exposure.

For the proposed grant period, we will prepare OP reporter molecules (e.g., radiolabel, biotin-linked) that will tag and assist with the identification of non-target (cholinesterase) proteins. To ensure the correct OP-adduct structures form in the interactions, dimethoxy and diethoxy-OP structures and reporter molecules will be reacted with purified AChE and the adducts confirmed by mass spectrometry (SA1). Next, new OP-protein adducts will be identified using a neuronal cell culture line (SH-SY5Y) that expresses AChE (SA2). The relative ease of treating cell culture, reproducibility, and identification of low abundance proteins are important model considerations. With the cell culture results to guide us, we will next identify OP-adducted proteins from saliva and blood. Key OP-adducted residues will be identified following protein digestion and MS analysis (SA3). Using information gathered on the structure of the OP-adducted peptide fragments, customized antibodies will be prepared and used to develop efficient diagnostic tests (ELISA) to identify and quantify OP-protein adducts.

In this presentation, we will report on our advances to produce recombinant acetylcholinesterase (rAChE), model reactions between DMOP and DEOP with AChE, synthesis of the various reporter groups and their interaction with rAChE. Also covered in this presentation will be our advances to identify OP-adducted proteins by mass spectrometry.