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Development of an immunoaffinity-HPLC-MS/MS diagnostic test for measuring exposure to Organophosphate pesticides using Agarose beads

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Statement of the Problem: Organophosphate (OP) pesticides are commonly used agricultural treatments with accidental exposure rates estimated at 25 million people per year in the developing world. Current diagnostic tests rely on non-specific activity assays of butyrylcholinesterase (BuChE), a serum protein and biomarker of OP compound exposure, or measurements of pesticides and their metabolites in blood and urine. Here, we present a specific and sensitive test to measure exposure to select OP pesticides as adducts to BuChE in plasma specimens.

Methodology: 100 μL aliquots of human plasma were incubated in spin columns containing protein-G coated agarose beads that were previously conjugated to antibodies against human BuChE. On-bead digestion was performed using pepsin to yield peptides corresponding to adducted or unadducted active sites of BuChE. Extracted samples were analyzed by HPLC-MS/MS for adducts to parathion and dichlorvos and for unadducted peptides. Samples were quantitated using a standard curve generated with reference materials. BuChE activity was measured both before and after extraction using the Ellman's assay to determine extraction efficiency.

Findings: BuChE inhibition was greater than 95% for plasma treated with parathion for 24 hours or plasma treated with dichlorvos for one hour. The extraction efficiency of BuChE was greater than 91.9% for plasma exposed to either parathion or dichlorvos, or for mixtures of these plasma samples. Unexposed plasma yielded an extraction efficiency of 80.0%. Bis-methoxy, bis-ethoxy, and aged ethoxy adducts ranged between 6.89 and 32.4 ng/mL in exposed plasma samples while unadducted BuChE peptides were present at 26.4 ng/mL in unexposed plasma samples.

Conclusion & Significance: We have developed a new HPLC-MS/MS method for measuring blood adducts to OP pesticides using agarose beads. The method is expected to be useful for retrospective analysis of exposures up to 21 days post-exposure and is accessible to most clinical laboratories worldwide.

Biography

Dr. Jennifer Knaack is an Assistant Professor in the Department of Pharmaceutical Sciences at the College of Pharmacy and Health Sciences, Mercer University, Atlanta, GA. She received her Ph.D. in Pharmaceutical Sciences from the University of Southern California, CA. Following her graduate studies, Dr. Knaack was a postdoctoral fellow at Lawrence Livermore National Laboratory, CA where she studied yeast metabolism using accelerator mass spectrometry. Dr. Knaack then spent four years at the Centers for Disease Control and Prevention in Atlanta, GA where she developed novel diagnostic methods for measuring human exposure to organophosphorus nerve agents and paralytic shellfish toxins. At Mercer University, Dr. Knaack runs the Analytical Toxicology and Clinical Diagnostics Laboratory where she develops of novel diagnostic methods for measuring exposure to environmental and synthetic toxins. She also serves as a chemical weapons expert and works with human rights groups to identify potential chemical weapons attacks.

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