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Immunomagnetic separation method for diagnosing organophosphate pesticides exposure in humans: A new application

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Statement of the Problem: Poisoning from exposure to organophosphate (OP) pesticides is a critical problem worldwide and is often associated with the application of pesticides or consumption of contaminated water or food. To date, the primary methods for measuring exposure to these compounds has been through urinalysis measurement of metabolites, measurement of free pesticides in blood, or measurement of the activity of butyrylcholinesterase (BuChE), a blood protein that adducts to OP compounds. We have adapted and validated a protein-G magnetic bead (MB) test, originally developed for measuring OP nerve agent exposure, for the diagnosis of OP pesticide exposure in humans.

Methodology: MBs were cross-linked to anti-BuChE antibodies and incubated with human plasma for the extraction of OP-adducted BuChE or un-adducted BuChE. Pooled human plasma was exposed to parathion or dichlorvos to form OP pesticide-adducted BuChE. Extracted BuChE was digested using pepsin and the resulting peptides were quantified by HPLC-MS/MS. The Ellman's assay was used to determine the percent inhibition and percent extraction of BuChE.

Findings: Untreated plasma, plasma treated with parathion for 1 hour and for 24 hours, and plasma treated with dichlorvos were extracted and analyzed. An additional pooled plasma sample containing a mixture of all exposed plasma samples was also analyzed. The percent inhibition of BuChE by OP pesticides in these samples was 0.00±0.00, 57.7±1.67, 96.4±1.30, 97.2±0.23 and 98.3±0.12%, respectively. The percent extraction efficiency was 91.0±2.15, 96.0±1.68, 99.4±0.08, 99.5±0.08 and 99.5±0.04%, respectively. Un-adducted and adducted BuChE peptide concentrations observed in each of these samples are shown in Figure 1.

Conclusion & Significance: We have successfully adapted and validated an MB method for diagnosing OP pesticide exposure in humans. In addition to providing specificity, this method is very sensitive, robust and high-throughput and can be used for diagnosing retrospective exposure of OP pesticides..

Biography

Amruta Indapurkar is a PhD Candidate in the Department of Pharmaceutical Sciences at Mercer University. She is currently working in a Analytical Toxicology laboratory on immunoprecipitation techniques, protein digestion methodologies and analysis of peptides using HPLC-MS/MS.

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