K43 Utilizing Protein Adducts as Exposure Markers for Nitrogen Mustard Chemical Warfare Agents

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After attending this presentation, attendees will have a better understanding of protein adducts and how these adducts can be used as exposure markers for chemical warfare agents.

This presentation will impact the forensic science community by demonstrating the advantages of protein adduct biomarkers as compared to more traditional methods of exposure determination.

This presentation investigates protein adduction by nitrogen mustard chemical warfare agents in the initial development of longer-term markers of human exposure to these compounds. Protein adducts are formed when electrophilic xenobiotics (either a parent compound or a metabolite) bind to nucleophilic amino acids in biological proteins. The three most important protein nucleophiles for xenobiotic adduction are thiol group of cysteine (Cys) and the amino groups of lysine (Lys) and histidine (His). The selectivity of adduction to these sites is in part dependent on the physicochemical nature of the electrophile in question.

The chemical warfare agents mechloroethamine (HN-2) and tris-(2-chlorethyl)amine (HN-3) are nitrogen mustards that can cause tissue blistering and ocular/respiratory damage following human inhalation and dermal exposure. Previous *in vitro* work in this study's laboratory demonstrated the ability of HN-2 and HN-3 to bind to Cys, Lys, and His residues in model peptides, in addition to His and Lys residues on purified hemoglobin (Hb) and Human Serum Albumin (HSA). The objective of this current work was to confirm and identify adducts on Hb and HSA after *in vitro* incubation with HN-2 and HN-3 in whole blood.

Adduct formation was induced by incubating human whole blood aliquots for 24h at 37°C with HN-2 and HN-3 at a molar excess as compared to protein nucleophile. Hb and HSA were extracted from whole blood via an initial centrifugation to separate erythrocytes (containing Hb) from plasma (containing HSA). Proteins were then extracted via precipitation with acidic acetone (Hb) or acidic alcohol (HSA). Extracted proteins were quantified using the Bradford protein assay, digested using trypsin, and the resulting tryptic peptides were separated and analyzed using Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS). Analysis was performed on an Agilent® 6530 Quadrupole Time-Of-Flight (QTOF) MS equipped with a 1290 Infinity® UHPLC and a ZORBAX Eclipse Plus C-18 Rapid Res HD (10mm x 2.1mm, 1.8µm particle size) column. Gradient elution of tryptic peptides with water and acetonitrile mobile phases containing 0.1% trifluoroacetic acid (TFA) was utilized. Agilent® Mass Hunter BioConfirm Software was used to identify specific sites of adduction. HN-2 and HN-3 adduction was observed on Hb and HSA, similar to what was seen in previous work using purified proteins incubated with these agents. Identified adducts were hydrolysis products of HN-2 and HN-3, most commonly found on His residues residing on the surface of both HSA and Hb. Adduction was confirmed using replicate samples, exact mass MS, and MS/MS analysis. Identified adducts were determined to be stable at 37°C for up to one week after incubation with HN-2 and HN-3.

The use of protein adducts can, in theory, allow for detection of HN-2 or HN-3 exposure long after urinary metabolites and/or parent compounds have been excreted from the body. This is of significance to the forensic community, as current methods focus on blood or urinary metabolites for exposure determination, which may suffer from certain drawbacks such as non-specificity and short detection window based on the half-life of the compounds of interest. It is of high importance to be able to identify previous exposure to these dangerous compounds, in terms of both victim exposure and in those individuals who may have been involved in unlawful handling or synthesis of these compounds.

Chemical Warfare Agents, Exposure Biomarkers, Nitrogen Mustards

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