K27 Ricin-Binding Proteins in Buccal Cells and Salivary Fluid

Oluseyi A. Vanderpuye, PhD, Albany State University, Forensic Science, 504 College Drive, Room 118, Hartnett Building, Albany, GA 31705*

After attending this presentation, attendees will learn how specific binding proteins for the toxin ricin can be identified in human buccal cells and cell free saliva.

This presentation will impact the forensic science community by demonstrating how the characterization of ricin binding proteins in salivary fluid and buccal cells proteins may facilitate discovery of methods for diagnosis of ricin poisoning and clarify additional details of mechanisms involved in ricin toxicity.

The plant protein ricin is one of the most poisonous known substances, is subject to biological and chemical weapons bans and is of concern as a tool of terrorists. There is no cure for ricin poisoning and diagnostic difficulty in distinguishing its effects from other harmful agents. Routes of exposure include ingestion, inhalation and injection. There are gaps in the knowledge of specific molecular identities of cell surface ricin-binding proteins. This research describes binding of ricin and the related lectin RCA-I to proteins in buccal cells and salivary fluid which are biological material that could be exposed to ricin during poisoning.

This study investigated if binding of ricin could be detected to buccal cell surfaces, salivary and buccal proteins and identification of molecular masses of ricin ligands. Whole saliva was collected by expectoration and salivary fluid and buccal cell fractions isolated by centrifugation. Ricin and RCA-I-binding proteins were detected by lectin blotting after SDS gel electrophoresis of saliva and buccal cell proteins and also measured by Enzyme-linked microtiter plate binding assays. Fluorescence microscopy with biotinylated ricin and RCA-I was used to visualize localization of ricin and RCA-I binding to buccal cell surfaces.

After electrophoresis, lectin blots identified a 170kDa buccal cell protein band in reduced samples that bound to ricin, binding was absent or decreased in non-reduced samples. Major ricin-binding proteins in salivary fluid included 170-150kDa, 75kDa, 50kDa, 40kDa and 25kDA molecules. Neuraminidase from Clostridium perfringens increased the binding of ricin to blots of salivary fluid proteins but had less effect on the binding of RCA-I. Treatment with neuraminidase from *Vibrio cholerae* did not affect the binding of ricin and RCA-I to buccal cell proteins in lectin blots. In fluorescence microscopy and microtiter plate binding assays, ricin bound only weakly to buccal cells in contrast to strong staining and binding seen with RCA-I.

Specific ricin and RCA-I –binding salivary and buccal cell proteins can be detected by lectin blotting after electrophoresis including a common 170kDa protein. There are differences in the reactivity patterns of the related molecules RCA-I and ricin with buccal cells and saliva, even though in the literature both are reported to bind to galactose- terminated oligosaccharide structures on proteins and glycolipids. Binding to buccal cells and salivary proteins could be relevant to the bioavailability of ricin or dose reaching other tissues in the event of poisoning by the oral route.

Ricin, Toxin, Saliva

 ** Presenting Author*