## A24 Ricin Bioavailability: Poor Binding to Human Serum Proteins and Toxicity Facilitation

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The goal of this presentation is to review how glycoproteins in human blood and serum are able to bind to ricin which is a highly toxic carbohydrate-binding protein. It was also an objective to show the capabilities of lectin blotting of electrophoresis gels in providing information on the characteristics of proteins found in human blood.

This presentation will impact the forensic science community by providing some insight into the extent of binding of serum glycoproteins by the toxin ricin. The poor binding of serum glycoproteins by ricin suggests that the glycoproteins in this body fluid may not effectively compete with cell surface proteins for ricin binding. This suggests that serum may not ameliorate ricin toxicity as might have been predicted because it contains glycoproteins that bind to the lectin RCA–I which has similar carbohydrate binding specificity to that of ricin.

It has been proposed that binding of ricin to proteins in human blood may affect its bioavailability, and, hence, its capacity to kill cells. Ricin binds to galactose residues in terminal beta glycosidic linkages on glycoproteins. Its binding to cell surface proteins and lipids which bear these structures allows ricin uptake by cells subsequently causing cell death.

Samples of 1mg/ml of the purified serum proteins, alpha 2 HS glycoprotein, transferring, and immunoglobulin G were mixed with an equal volume of SDS gel electrophoresis buffer which contained 10mM dithiothreitol and denatured by boiling. Six different human serum samples were diluted 20-fold in SDS gel electrophoresis buffer which contained the reducing agent dithiothreitol and the proteins were also denatured by boiling. Sample volumes of 20ul were electrophoresed in 4-20% gradient polyacrylamide gels and electrotransferred to nitrocellulose. Blots were probed with the following biotinylated lectins: ricin; ricinus communis agglutinin-II; sambucus nigra agglutinin; and, concanavalin A. The blots were developed by using streptavidin-alkaline phosphatase which was followed by the chromogenic blot substrate combination of 5-bromo-4 chloro-3 indolyl phosphate and nitroblue tetrazolium.

Six serum samples and the purified human proteins, transferrin, immunoglobulin G and alpha2 HS glycoprotein were separated by SDS gel electrophoresis. Total protein staining of the electrophoresis gel with Coomassie Brilliant Blue dye revealed the typical pattern of protein bands for the serum samples. A predominant protein band of approximately 66,000 Daltons corresponding to serum albumin was present in all serum samples. Strong protein bands at 50,000 and 25,000 Daltons (immunoglobulin G) and 75,000 Daltons (transferrin) were also present in the serum samples by comparison with the purified proteins which were electrophoresed on the same gel. Alpha2 HS glycoprotein is heavily glycosylated and does not stain well with Coomassie Blue but was detected in the sample of the purified protein as bands at a position of 50,000 Daltons. The carbohydrate structures present on the purified proteins have been studied in detail by other investigators and those on transferrin and immunoglobulin G would be expected to be able to bind to ricin by blotting electrophoresis gels. Other lectins of known carbohydrate-binding specificity were used in this study as controls to show that the lectin blotting technique was working properly. Concanavalin A (Con A), sambucus nigra agglutinin (SNA), and ricinus communis agglutinin have binding specificities that allowed prediction of their binding to the purified proteins and could be used to help interpret the results with ricin.

As expected, Con A bound to purified transferrin and the 50,000 Dalton polypeptide of immunoglobulin G which contains high mannose and biantennary structures but poorly to alpha2 HS glycoprotein. SNA binds to carbohydrates that contain alpha 2-6 linked sialic acid and to purified transferrin and alpha2 HS glycoprotein but not to immunoglobulin G which usually contains little or no sialic acid. Sialic acid molecules are sometimes linked to galactose on proteins and would prevent binding of ricin to such glycoproteins. From these results, it will be possible to later perform experiments involving enzymatic removal of sialic acid to see how ricin binding is affected and to use SNA as a control. RCA-I is a lectin that binds terminal beta-linked galactose on glycoproteins and might be expected to bind the same glycoproteins as ricin. RCA-I bound strongly to many serum proteins and also to transferrin and to the 50,000 Dalton polypeptide of immunoglobulin G, both of which have been characterized as containing terminal beta-linked galactose. RCA-I did not bind to alpha2 HS glycoprotein which instead is known to contain large amounts of sialic acid. Surprisingly, ricin exhibited comparatively weak binding to immunoglobulin G and transferrin and serum proteins and no binding to alpha2 HS glycoprotein.

From these results it was concluded that ricin showed poor binding to serum proteins whereas the related protein RCA-I, which comes from the same plant, bound strongly to serum proteins. The poor serum protein binding by ricin could help explain its extreme toxicity because proteins in blood would compete poorly with ricin for binding to cells.

**Ricin, Serum, Blood**

 ** Presenting Author*