Molecular Toxicology of Low-Level Exposure to Nerve Agents

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Introduction:

Gene and protein expression are sensitive indicators of toxicant exposure, disease states, and cellular metabolism, giving a rapid and detailed picture of cellular reaction to the environment. Not only can measuring gene and protein expression levels yield a wealth of information about mechanism of action, but the groups of genes and proteins altered in expression can be used as expression "biomarkers" for a particular toxicant exposure. Recently, many researchers have begun using transcript and protein expression markers in order to quickly screen for toxicity or the presence of a disease state in a tissue.

Problem:

Over the past more than 75 years a great body of knowledge has been collected on the acute effects of nerve agents. However, not until after the first Gulf War, and the appearance and controversy of Gulf War Syndrome, has there been much research examining the effects of low level and chronic exposure to these and other chemicals of military significance. Currently, there are no expedient field assays that can determine whether someone has been exposed to a low-level of chemical warfare agent (CWA). Furthermore, if it were known that a person had been exposed, there is a great paucity of information regarding the potential for transient or persistent neurological, cardiac, or other type of injuries.

In order to begin to address the complex question of consequences of low level CWA exposure, our laboratory is investigating the hypothesis that there are distinct and persistent gene and protein expression changes occurring as a result of inhalation of low-level nerve agent vapor (i.e. sarin, cyclosarin, and VX). Presently, our laboratory is investigating gene expression changes using both transcriptomic (DNA microarray and RT-PCR) and proteomic (1-D and 2-D PAGE and LC-MS-MS) techniques to identify mechanisms of toxicity and biomarkers of agent exposure in the brain, heart, blood, and hair follicle of model animals exposed to nerve agent vapor. These tissues are being examined at a wide range of post-exposure time points (e.g. one day-two months) in order to reveal both transient and persistent toxic molecular effects.

Summary of talk:

This talk will provide an overview of the current state of molecular toxicology, highlighting some of the different problems that have driven the development of the research technologies being used today. Background on the unique questions regarding the possible consequences of low-level CWA exposure will also be presented. A discussion on how our laboratory is attempting to answer some of these questions will be punctuated with fundamental explanations of the technologies being used to collect and mine the data. Lastly, there will be a discussion of the many potential practical outcomes of this type of molecular toxicological investigation as well as the potential ethical and legal implications of the type of knowledge that may be gained from molecular toxicological investigations.

Keywords:

Transcriptomics: The study of transcription regulation and the products of transcription (mRNA transcripts), using a variety of techniques to quantify specific transcripts. The process

whereby mRNA (messenger RNA, encoding specific gene) is copied from DNA is called transcription. Two such techniques are:

DNA microarray: An array of 1000s to tens of 1000s of short DNA fragments attached to a solid substrate (usually a small square of glass or plastic) to which labeled mRNA is hybridized. Relative expression of specific genes can be determined by comparing the intensity of the hybridization of the transcripts (from either control or experimental sample) to gene-specific spots on the array (units: fold increase or decrease). If the entire genome has been sequenced, and its entirety has been included on the microarray, it is possible to measure the entire complement of transcripts present in a sample at a given point in time.

RT-PCR: Reverse-transcription polymerase chain reaction is a technique that allows one to measure the relative quantity of a specific transcript in a sample. It "reverse transcribes" the mRNA to cDNA, then amplifies the cDNA products by standard PCR. The relative level of product created by that reaction can be compared to a reference (or control) sample, and "up" or "down" regulation of a transcript can be determined.

Proteomics: This "omics" term encompasses many techniques used to study the expression, regulation, location, and modification of proteins. It is often used when describing efforts to characterize the entire complement of proteins (proteome) of a cell or organism at a given point in time. Two such techniques are:

1-D and 2-D PAGE: One-dimensional polyacrylamide gel electrophoresis separates proteins and polypeptides based on shape and charge. From the distance the protein travels in the gel, relative to a mass ladder standard, the approximate mass can be deduced (units: Daltons). The two-dimensional PAGE separates the proteins first by isoelectric point (pI), then by shape and charge in the second dimension. The 2-D PAGE produces data that aids in the identification of small protein expression level changes and post-translational modifications of individual proteins (units: Daltons and pI). Isoelectric point (pI) is the pH at which the protein has a neutral charge.

LC-MS-MS: Liquid chromatography followed by tandem mass spectrometry. The LC step separates the mixed protein samples by size. The tandem MS step, preceded by tryptic digestion of the proteins into their component peptides and followed by peptide signature matching informatics, provides identification of the proteins that comprise the sample.