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Biology

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UNIT 5: Respiration, Internal Environment, Coordination and Gene Technology

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Assessment of food toxicology by Alexander Gosslau

1. The history of food toxicity might have started as early as Hippocrates made the statement "Let food be thy medicine and medicine thy food" which presaged the modern science by over two millennia ago. With the development of modern biochemistry, molecular biology, cell culture techniques, computer science and bioinformatics, it has been possible to identify and characterize potential toxicants in food.

2. There are two different related areas in the measurement of toxicants and toxicity in food: (1) actual measurements of the effects of toxicants in different models ranging from *in vitro* biochemical systems, cell-based *in vitro* systems, animal *in vivo* models to clinical settings analyzing systemic or organ-specific toxicity and (2) assessment and/or predictions of potential toxicants in food.

3. The mechanisms of toxicant effects are multifactorial interacting intrinsically and extrinsically with key molecules which play major roles in cell integrity, metabolism, signaling pathways, gene expression and translation. For a variety of toxicants their effects appear to converge on the generation of electrophilic species (ES) leading to oxidative stress and chronic inflammation. Oxidative reactions induced by toxicants lead to an accumulation of damaged macromolecules thus harming cells, tissues and organs. Therefore, toxicants may play central roles in cell death, chronic inflammation, aging and degenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases, as well as multiple sclerosis, myocardial infarction, arteriosclerosis, diabetes, rheumatoid arthritis, sterility, cataracts and many others.

4. For *in vitro* assessment a variety of biochemical systems have been developed to analyze damaging effects on integrity or activity of key biomolecules. Such molecules are important in cell integrity, metabolism, signaling pathways, as well as gene expression and translation. The list of affected molecules is extensive and includes enzymes, receptors, membrane lipids, nucleic acids and/or factors involved in gene expression. On a cellular level, a variety of viability assays are routinely used to quantify effects of potential food toxicants for extrapolation of a range of dosages used for maximal tolerated concentrations for *in vivo* animal models and also clinical settings. *In vivo* rodent models still appear to be the gold standard for toxicity assessment but there are limitations of such traditional testing such as high costs, low throughput readouts, inconsistent responses, ethical issues and concerns of extrapolability to humans.

5. Another refinement in toxicity assessment is the installation of alternative lower hierarchy surrogate animal models such as zebrafish (*Danio rerio*), fruit flies (*Drosophila melanogaster*) or nematodes (*Caenorhabditis elegans*). These models offer an advantage in terms of ethical concerns, high throughput and genetic manipulation over traditional rodent models. The value of using alternative sub-mammalian vertebrate and invertebrate models became evident by the surprising discovery of the high degree of homology of genes between humans and zebrafish, fruit flies or nematodes.

6. Although the mechanisms leading to toxic effects in humans are multifactorial, the majority of toxic effects appear to converge on the generation of free radicals. Different electrophilic species (ES) such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) are capable of oxidizing virtually all biomolecules. Whereas a variety of toxicants generate ES directly, others induce a secondary response leading indirectly to generation of ES by immunocompetent leukocytes which play a key role in the inflammatory cascade. ES are also involved in the modulation of gene expression by interfering with transcription factors and/or DNA which can lead to mutations and carcinogenesis. The accumulation of damage to membrane lipids, cellular proteins, carbohydrates as well as nucleic acids harm the functioning of cells, tissues and organs. These and other observations strengthen the hypothesis that toxicants leading to oxidative stress and chronic inflammation play central roles in cell death, aging and degenerative diseases.

7. The oxidation of lipids, proteins, nucleic acids and carbohydrates generate a variety of damaging breakdown products which thus can lead to the onset of many degenerative diseases.
Lipid peroxidation of cell structures containing lipids can lead to the generation of different toxic products including alcohols, ketones, alkanes, aldehydes and ethers which have the potential to contribute to cell damage, necrosis or apoptosis. Nucleic acids are delicate targets of ES leading to mutations. Damage of nucleic acids by ES may result in single and double strand breaks, DNA–DNA, DNA–protein, DNA–lipid adducts or numerous base modifications. Mitochondrial DNA (mtDNA) is particularly susceptible to oxidative damage because of the absence of associated histones, an incomplete mitochondrial DNA repair system and the generation of free radicals through electron leakage from the respiratory chain.

8. Testing whether a chemical can modulate the activity of particular enzyme or binding affinities to a particular receptor or other biomolecule is the most direct way to gain mechanistic insights into action at the molecular level. There are different biochemical *in vitro* assays which analyze the integrity or mutation of DNA and RNA, membrane lipids, as well as the binding and activity of various receptors, enzymes involved in signaling transduction, drug or neurotransmitter metabolism and many others.

9. The use of cellular models provides a much higher level of complexity than simple biochemical assays. A huge number of human cell lines are available and a variety of different cell-based *in vitro* assays have been developed for screening of food toxicants.

10. For general assessment of cytotoxicity an indirect measure of cell viability is usually performed and several cellular bioassays are routinely used integrating different cytotoxicity endpoints such as membrane leakage or cellular activity. Whereas the trypan blue, propidium iodide, crystal violet or lactate dehydrogenase assays are analyzing membrane integrity based on exclusion, other viability assays such as the neutral red, alamar blue or MTT assay are metabolic measures of cellular activity. Inhibitory concentrations (IC values) obtained by viability assays are then used for initial dose selection in testing on animals and humans.

    In addition to the measurement of dyes, activity analysis of enzymes is also an established technique used to determine membrane integrity. Leakage of intracellular enzymes such as lactate dehydrogenase (LDH) or others into the extracellular medium is thus employed as indicator of cell membrane damage.

    Damage of mitochondria is a major contributor to organ toxicity, such as of the liver, kidney, heart, muscle and the central nervous system, and mitochondrial dysfunction is increasingly implicated in a growing list of degenerative diseases.

11. Recently, stem cell-based assays are being discussed as source for various toxicological applications thanks to the Nobel Prize-winning discovery of how to reprogram ordinary somatic cells to behave like embryonic stem cells. Human-induced pluripotent stem cells (iPSCs) allow assays to consider an individual's genetic background and potential epigenetic influences that affect the variability of the toxicity response. Stem cell-based models are also of particular interest for toxicity measurements which either lack extrapolability in rodent models such as for genotoxicity, cardiotoxicity, respiratory toxicity or for different stages of disorders which largely remain unknown such as neurological disorders (depression, anxiety, Alzheimer's and Parkinson's disease), autoimmune diseases (multiple sclerosis, type I diabetes, asthma), systemic infection, cancer and others.

12. While complex cell culture systems can provide unique insights into *in vivo* toxicology, they will never completely model the higher level interactions present in an intact organism. Therefore, the gold standard for toxicity assessment has been *in vivo* toxicology, where a particular molecule or complex food ingredients are given to animals to evaluate acute, subacute and chronic effects.

13. The majority of animals used are rodents and to derive statistically significant results the numbers of animals needed for testing are enormous with an estimation of 7000 animals and tens of millions of dollars for each test compound in the pharmaceutical industry. Although the numbers of animals involved in food toxicity screening are decreasing, the numbers of compounds or food ingredients to be tested as well as the costs of the current *in vivo* assessment systems are exploding.

14. More recently, the zebrafish *D. rerio* has been used as a vertebrate model organism for a wide variety of research including drug discovery and toxicology. The increased usage of zebrafish as *in vivo* model system reflects the striking similiar toxicity profile between humans and zebrafish due to substantial physiological, anatomic and genetic homology. The zebrafish model is also amenable to gene manipulation, is low in cost, has a short generation time and is particularly well suited for high-throughput screening as well as microarray and proteomic studies. Since zebrafish larvae are transparent they are ideal for studies on organ morphology by *in vivo* imaging techniques in addition to more detailed studies by immunohistochemistry or *in situ* hybridization.

15. Toxicity assessment in humans involves different fields such as clinical, forensic, environmental and regulatory toxicology. A systemic determination of toxicants in body tissues is usually obtained by biopsy or by analyzing body fluids such as blood and urine.

16. A great deal of knowledge on toxicity in humans has been obtained by post mortem molecular and anatomic analysis of cells, tissues and organs. Forensic toxicology is very related to toxicologic pathology but focusing more on the application to the purposes of the law. By virtue of advances in nanotechnology and its application in food industry, the newly created discipline of nanotoxicology investigates safety or potential hazards of nanoparticles. Another dimension refers to genetically modified organisms (GMO) or genetically modified food (GMF) as potential sources of toxicity. All the different disciplines of toxicity assessment in humans are not mutually exclusive but rather highly interconnected. The goal is to identify and understand the molecular mechanisms of toxicants causing adverse effects in order to ultimately prevent their intake thus increasing food safety.