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TOXICOLOGY

NEWSLETTERS

"Life cannot have had a random beginning ... The trouble is that there are about 2000 enzymes, and the chance of obtaining them all in a random trial is only one part in 10^40,000, an outrageously small probability that could not be faced even if the whole universe consisted of organic soup."

- Fred Hoyle, Evolution from Space

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Chemical-Induced Cellular Toxicity Mechanisms

K.S. Rao M.V.Sc. Ph.D., DABT
Eurofins Advinus Limited, Bangalore

1. Preamble

In toxicology, dose-response relationship for toxic agents has become increasingly complex. This relationship, which has long been regarded as part of the foundation of toxicology, can be straight forward for a measurable acute response. Often, however, the exposure to a chemical does not result in overt tissue injury but may affect cell functions in a more subtle way, thereby increasing the susceptibility to other forms of damage. The problem becomes even more intricate when assessing the potential risk of chronic exposure to an agent that has no obvious acute effect but may still have long-term consequences. The molecular mechanisms that result in acute effects are often distinct from those responsible for chronic toxicity.

Whether a cell survives or dies in the presence of a chemical insult is often determined by proliferative status, repair enzyme capacity, and the ability to induce proteins that either promote or inhibit the cell death process. *Understanding the underlying mechanisms allows for a better prediction of the consequences of exposure to low levels of toxicants and a safer assessment of the risks associated with environmental pollution.*

2. Mechanistic Toxicology

Mechanistic toxicology is the study of how chemical or physical agents interact with living organisms to cause toxicity. Knowledge of the mechanism of toxicity of a substance enhances the ability to prevent toxicity and design more desirable chemicals; it constitutes the basis for therapy upon overexposure and frequently enables a further understanding of fundamental biological processes. *Different areas of toxicology include mechanistic, descriptive, regulatory, forensic and environmental toxicology. All of these benefit from understanding the fundamental mechanisms of toxicity.*

Mechanistic understanding helps the governmental regulator to establish legally

Editor
Alex Thomas,
M.Pharm., (Ph.D.)
Bioneeds India Pvt Ltd.
+91-974-545-0045
alexthomas.a@gmail.com

binding safe limits for human exposure. It helps toxicologists in recommending courses of action regarding clean-up or remediation of contaminated sites and, along with physical and chemical properties of the substance or mixture, can be used to select the degree of protective equipment required. *Mechanistic knowledge is also useful in forming the basis for therapy and the design of new drugs for the treatment of human disease.*

If the mechanism of toxicity is understood, descriptive toxicology becomes useful in predicting the toxic effects of related chemicals. Prudent decisions based on animal studies and human experience are used to establish safe exposure levels. *Traditionally, a margin of safety was established by using the "no adverse effect level" or a "lowest adverse effect level" from animal studies (using repeated-exposure designs) and dividing that level by a factor of 100 for occupational exposure or 1,000 for other human environmental exposure.* The success of this process is evident from the few incidents of adverse health effects attributed to chemical exposure in workers where appropriate exposure limits had been set and adhered to in the past. In addition, the human lifespan continues to increase, as does the quality of life. Overall the use of toxicity data has led to effective regulatory and voluntary control. Detailed knowledge of toxic mechanisms will enhance the predictability of newer risk models currently being developed and will result in continuous improvement.

3. How to Study Mechanisms of Toxicity?

Mechanistic studies start with a descriptive toxicological study in animals or clinical observations in humans. Ideally, animal studies include careful behavioral and clinical observations, careful biochemical examination of elements of the blood and urine for signs of the adverse function of major biological systems in the body, and post-mortem evaluation of all organ systems by microscopic examination to check for injury.

Understanding mechanisms of toxicity are the art and science of observation, creativity in the selection of techniques to test various hypotheses, and innovative integration of signs and symptoms into a causal relationship. *Mechanistic studies start with exposure, follow the time-related distribution and fate in the body (pharmacokinetics), and measure the resulting toxic effect at some level of the system and at some dose level.* Different substances can act at different levels of the biological system in causing toxicity.

A. Route of Exposure

The route of exposure in mechanistic studies is usually the same as for human exposure. *The route is important because there can be effects that occur locally at the site of exposure in addition to systemic effects after the chemical has been absorbed into the blood and distributed throughout the body.*

A simple yet cogent example of a local effect would be irritation and eventual corrosion of the skin following application of strong acid or alkaline solutions designed for cleaning hard surfaces. Similarly, irritation and cellular death can occur in cells lining the nose and/or lungs following exposure to irritant vapors or gases such as oxides of nitrogen or ozone. Following absorption of a chemical into the blood through the skin, lungs or gastrointestinal tract, the concentration

in any organ or tissue is controlled by many factors which determine the pharmacokinetics of the chemical in the body. The body has the ability to activate as well as detoxify various chemicals as noted below.

B. Impact of Pharmacokinetics on Toxicity

Pharmacokinetics describes the time relationships for chemical absorption, distribution, metabolism, and elimination from the body. Relative to mechanisms of toxicity, these pharmacokinetic variables can be very important and, in some instances, determine whether toxicity will or will not occur. For instance, if a material is not absorbed in a sufficient amount, systemic toxicity will not occur. Conversely, a highly reactive chemical that is detoxified quickly by digestive or liver enzymes may not have the time to cause toxicity. Some polycyclic halogenated substances and mixtures, as well as certain metals like lead, would not cause significant toxicity if excretion were rapid, but accumulation to sufficiently high levels determines their toxicity since excretion is not rapid. Fortunately, most chemicals do not have such long retention in the body. *The rate of elimination from the body and detoxication is frequently referred to as the half-life of the chemical, which is the time for 50% of the chemical to be excreted or altered to a non-toxic form.*

However, if a chemical accumulates in a cell or organ, that may signal a reason to further examine its potential toxicity in that organ. More recently, mathematical models have been developed to extrapolate pharmacokinetic variables from animals to humans. These pharmacokinetic models are extremely useful in generating hypotheses and testing whether the experimental animal may be a good representation for humans.

C. Various Levels and Systems Affected

Toxicity can be described at different biological levels. Injury can be evaluated in the whole animal, the organ system, the cell or the molecule. Organ systems include the immune, respiratory, cardiovascular, renal, endocrine, digestive, musculoskeletal, blood, reproductive, and central nervous systems. Some key organs include the liver, kidney, lung, brain, skin, eyes, heart, testes or ovaries, and other major organs. *At the cellular/biochemical level, adverse effects include interference with normal protein function, endocrine receptor function, metabolic energy inhibition, or xenobiotic enzyme inhibition or induction*. Adverse effects at the molecular level include alteration of the normal function of DNA-RNA transcription, of specific cytoplasmic and nuclear receptor binding, and of genes or gene products. Ultimately, dysfunction in a major organ system is likely caused by a molecular alteration in a particular target cell within that organ. However, it is not always possible to trace a mechanism back to a molecular origin of causation, nor is it necessary. However, knowledge about the specific mechanism of toxicity increases the predictive value and accuracy of extrapolation to other chemicals.

D. Types of Mechanisms of Toxicity

Mechanisms of toxicity can be straightforward or very complex. Frequently, there is a difference among the type of toxicity, the mechanism of toxicity, and the

level of effect, related to whether the adverse effects are due to a single, acute high dose (like an accidental poisoning), or a lower-dose repeated exposure (from occupational or environmental exposure).

a. Acute Toxicity Mechanisms

i. Simple asphyxiants The mechanism of toxicity for inert gases and some other non-reactive substances is lack of oxygen (anoxia). These chemicals, which cause a deprivation of oxygen to the central nervous system (CNS), are termed simple asphyxiants.

ii. Chemical Asphyxiants Carbon monoxide (CO) competes with oxygen for binding to hemoglobin and therefore deprives tissues of oxygen for energy metabolism; cellular death can result. Another potent chemical asphyxiant is cyanide. The cyanide ion interferes with cellular metabolism and utilization of oxygen for energy. Treatment with sodium nitrite causes a change in hemoglobin in red blood cells to methemoglobin. Methemoglobin has a greater binding affinity to the cyanide ion than does the cellular target of cyanide. Consequently, the methemoglobin binds the cyanide and keeps the cyanide away from the target cells. This forms the basis for antidotal therapy.

iii. Central Nervous System (CNS) Depressants Acute toxicity is characterized by sedation or unconsciousness for several materials like solvents which are not reactive, or which are transformed into reactive intermediates. It is hypothesized that sedation/anesthesia is due to an interaction of the solvent with the membranes of cells in the CNS, which impairs their ability to transmit electrical and chemical signals.

iv. Skin Effects Adverse effects to the skin can range from irritation to corrosion, depending on the substance encountered. Strong acids and alkaline solutions are incompatible with living tissue and are corrosive, causing chemical burns and possible scarring. Scarring is due to the death of the dermal, deep skin cells responsible for regeneration. Lower concentrations may just cause irritation of the first layer of skin.

v. Skin Sensitization Sensitization occurs when 2,4-dinitrochlorobenzene binds with natural proteins in the skin and the immune system recognizes the altered protein-bound complex as a foreign material. In responding to this foreign material, the immune system activates special cells to eliminate the foreign substance by a release of mediators (cytokines) which cause a rash or dermatitis. Immune sensitization is very specific to the particular chemical and takes at least two exposures before a response is elicited. The first exposure sensitizes (sets up the cells to recognize the chemical), and subsequent exposures trigger the immune system response.

vi. Lung Sensitization An immune sensitization response is elicited by toluene diisocyanate (TDI), but the target site is the lungs.

TDI over-exposure in susceptible individuals causes lung edema, bronchial constriction, and impaired breathing.

vii. Eye Effects Injury to the eye ranges from reddening of the outer layer to cataract formation of the cornea to damage to the iris.

b. Sub-Chronic and Chronic Toxicity Mechanisms

i. Anticholinesterase Inhibition The primary mechanism of action of organophosphates is the inhibition of acetylcholinesterase (AChE) in the brain and peripheral nervous system. AChE is the normal enzyme that terminates the stimulation of the neurotransmitter acetylcholine. Slight inhibition of AChE over an extended period has not been associated with adverse effects. At high levels of exposure, inability to terminate this neuronal stimulation results in overstimulation of the cholinergic nervous system. Cholinergic overstimulation ultimately results in a host of symptoms, including respiratory arrest, followed by death if not treated. The primary treatment is the administration of atropine, which blocks the effects of acetylcholine, and the administration of pralidoxime chloride, which reactivates the inhibited AChE.

ii. Metabolic Activation Many chemicals, including carbon tetrachloride, chloroform, acetylaminofluorene, nitrosamines, and paraquat are metabolically activated to free radicals or other reactive intermediates which inhibit and interfere with normal cellular function.

iii. Cancer Mechanisms Cancer development is a multi-stage process, and critical genes are key to different types of cancer. Alterations in DNA (somatic mutations) in a number of these critical genes can cause increased susceptibility or cancerous lesions. Exposure to natural chemicals (in cooked foods like beef and fish) or synthetic chemicals (like benzidine, used as a dye) or physical agents (ultraviolet light from the sun, radon from the soil, gamma radiation from medical procedures or industrial activity) are all contributors to somatic gene mutations. It is clear that genetics is an important factor in cancer, since genetic disease syndromes such as xeroderma pigmentosum, where there is a lack of normal DNA repair, dramatically increase susceptibility to skin cancer from exposure to ultraviolet light from the sun.

iv. Reproductive Mechanisms It is known that certain viruses (such as rubella), bacterial infections and drugs (such as thalidomide and vitamin A) will adversely affect development. Abnormal developmental effects in animal tests with ethylene glycol are attributable to maternal metabolic acidic metabolites. This occurs when ethylene glycol is metabolized to acid metabolites including glycolic and oxalic acid. Conclusion

v. Signaling Pathways With many types of injury, mitochondrial respiration and oxidative phosphorylation are rapidly affected. In some cells, this stimulates anaerobic glycolysis, which can maintain ATP, but with many injuries, this is inhibited. The lack of ATP

results in failure to energize several important homeostatic processes, in particular, control of intracellular ion homeostasis. This results in rapid increases of [Ca2+]i, and increased [Na+] and [Cl-] results in cell swelling. Increases in [Ca2+]i result in the activation of a number of other signaling mechanisms, including a series of kinases, which can result in increased immediate early gene transcription. Increased [Ca2+]i also modifies cytoskeletal function, in part resulting in bleb formation and in the activation of endonucleases, proteases, and phospholipases. These seem to trigger membrane damage through protease and lipase activation, direct degradation of DNA from endonuclease activation, and activation of kinases such as MAP kinase and calmodulin kinase, which act as transcription factors.

E. Methods of Target Organ Toxicity Studies

Target organs may be studied by exposure of intact organisms and detailed analysis of function and histopathology in the target organ, or by in vitro exposure of cells, tissue slices, or whole organs maintained for short- or long-term periods in culture.

In general, target organ toxicity studies share the following common characteristics: *detailed histopathological examination of the target organ, including post mortem examination, tissue weight, and examination of fixed tissues; biochemical studies of critical pathways in the target organ, such as important enzyme systems; functional studies of the ability of the organ and cellular constituents to perform expected metabolic and other functions; and analysis of biomarkers of exposure and early effects in target organ cells.*

a. Oxidative Stress

There are several mechanisms that are thought to be central to toxicological injury, including impaired lysosomal function, membrane changes, and oxidative stress. It is now widely accepted that Ca^{2+}-homeostasis in the cell and Ca^{2+}-mediated cell functions are critical targets for numerous pathophysiological processes including toxicant-induced cell death. Many classes of pharmaceuticals and other chemicals (e.g., metals, pesticides, and solvents) impair the calcium messenger system. Disturbances in intracellular Ca^{2+} homeostasis and sustained increase in cytosolic Ca^{2+} cause cell death by the disruption of the plasma membrane, cytoskeleton, endoplasmic reticulum, and mitochondria. In addition, chemicals (alkylating or arylating agents) can be toxic and may induce cell death through an initial DNA damage or by apoptosis (receptor-mediated programmed cell death). In cell injury caused by chemical toxicants, cellular accumulation of Ca^{2+} and the generation of oxygen free radicals damage cellular components, particularly mitochondrial membranes. Indeed Ca^{2+} potentiates oxygen free radical injury to renal mitochondria, and the result of this detrimental interaction could be due, in part, to the activation of phospholipase A2.

Lipid peroxidation has been suggested as one of the possible mechanisms whereby chemicals may produce membrane damage and cell death. Free radicals, generated either directly by the metabolism of a chemical or from the reduction of oxygen (forming O_2^-, H_2O_2, and OH*), can initiate lipid peroxidation *via* hydrogen abstraction from polyunsaturated fatty acids. This interaction will form lipid peroxy-radicals and lipid hydroxy-peroxides, propagating the chain reaction. Such a chain reaction may destroy cellular membranes and thereby result in

increased plasma membrane permeability or altered fluidity and cell death. Lipid peroxidation may also cause cell death through the formation of potentially toxic lipid metabolites (such as hydroxy-alkenals). However, several lines of evidence indicate that lipid peroxidation is most often independent (or is a consequence rather than the cause) of cell death. One or more of these mechanisms of cellular injury could closely interact.

Proximal renal tubular cells are particularly vulnerable to the toxic action of chemicals, owing to their high energy demand (such as reabsorptive and secretory functions). Redox-active agents may cause extensive oxidation of GSH to oxidized glutathione (GSSG). Under such conditions, often referred to as "oxidative stress", reduction of GSSG back to GSH by the NADPH-dependent GSSG reductase is lower than the rate of GSH oxidation. This may lead to glutathione depletion and cause oxidation of cellular enzymes, depletion of cellular ATP, and loss of mitochondrial function.

Reactive electrophilic metabolites are known to bind covalently to tissue proteins, and it has been suggested that cell injury and death are a consequence of the interaction of such reactive intermediates with critical cellular molecules. Free sulfhydryl groups are involved in the catalytic activity of many proteins. Modification of such sulfhydryl groups by covalent binding or by oxidation may inactivate critical enzymes and lead to cell death. For some chemicals, the loss of protein sulfhydryl groups results mainly from a reversible oxidative process, which leads to the formation of disulfide cross-links or mixed disulfides with another protein or GSH. Enzymes involved in Ca^{2+} homeostasis may be one example of such critical cellular target molecules for alkylating/arylating or oxidizing metabolites.

F. Factors that Modify Cellular Injury by Toxins

a. Cellular Transport and Accumulation

Drugs and other chemicals including metals may be transported across proximal tubular cells, i.e., from renal capillaries across tubular cells to be excreted in the tubular lumen or *vice versa* (absorption). Many organic anions are excreted against concentration gradients at rates that exceed glomerular filtration. This implies an active carrier-mediated transport process. Such a process requires energy obtained from oxidative metabolism located in mitochondria. An active process for transporting solutes in renal tubular cells has certain implications concerning the susceptibility of tubular cells to effects of toxins. *If cationic drugs or chemicals are actively transported, there is the immediate problem of competition with the transport of essential cations. Active transport with the capability of concentrating absorbed material may concentrate potential nephrotoxins as well as essential solutes in the renal cortex*. The same toxins that impair energy metabolism will impede the cellular transport of essential solutes. Other toxic substances may be concentrated in the medulla or the papillae, probably as a consequence of the physiological mechanism that concentrates urine. The renal accumulation of chemicals such as gentamicin, cephaloridine, or cadmium is well documented.

b. Metabolic Degradation

Metabolic degradation or transformation most often occurs in the liver, but many of the same enzyme systems are present in the kidney as well. The metabolism of drugs and chemicals within the kidney may result in substances that are either toxic. Those drugs and chemicals that are metabolized by the mixed-function oxidase system have received the most attention. *For example, several*

chlorinated alkyl hydrocarbons of low relative molecular mass, such as carbon tetrachloride and trichloromethane, may be transformed into reactive, toxic products that bind covalently to renal tissue, producing membrane injury. In addition, low-level exposure to other substances, such as polychlorinated biphenyls (PCBs), that activate the enzyme systems may enhance the production of toxic products. Similarly, pretreatment with phenobarbital enhances the activity of mixed function oxidase enzymes and, hence, the toxicity of compounds like methoxyflurane whose metabolic products are fluoride and oxalate, two substances potentially toxic to the kidney. The fluoride ion is toxic to cell membranes, whereas oxalate may precipitate within the lumen of nephrons. On the other hand, phenobarbital reduces the renal toxicity of DBCP due to an increase in its detoxication in the liver.

c. Intracellular Protein Binding

The intracellular concentration of toxins may be influenced by protein binding. The soluble cytoplasmic protein, metallothionein, and insoluble acidic protein complexes forming nuclear inclusion bodies are examples of a phenomenon that concentrates two different groups of metals.

Metallothionines are proteins of low relative molecular mass(6000-7000 Daltons) characterized by a high cysteine content (23-33%),a complete lack of aromatic acids, and a high content of heavy metals(7-12 metal atoms/molecule of protein). *Metallothionines can bind several essential or non-essential heavy metal ions including zinc, copper, cadmium, mercury, silver, gold, and cobalt. The metal ions are bound exclusively through thiolate coordination complexes, which involve all the cysteine residues (20 in rat liver metallothionein) located in two domains (alpha and ß domains).* Metal ions that can bind to metallothionines can also, to variable extents, promote the transcriptional activity of metallothionein genes. In the kidney, Cd^{2+} and Hg^{2+} are the best inducers of metallothionein synthesis. Metallothionein synthesis can also be induced by various stresses (e.g., tissue injury, food restriction, infections). Not all the biological functions of metallothionein have been fully elucidated. They probably include protection against and detoxification of heavy metals, regulation of the metabolism and possibly the function of essential elements such as copper and zinc, and a protective response to various stresses by altering zinc distributions between tissues and within cells (e.g.,macrophages) and by acting as a free radical scavenger.

Lead and bismuth accumulate in renal tubular cells bound to a complex of acidic proteins that form morphologically discernible inclusion bodies. As with metallothionein, the sequestering of toxic metals by the protein complex is thought to reduce the intracellular toxicity of these metals.

d. Membrane Reactions and Pinocytosis

Macromolecular substances are transported by pinocytosis and included in intracellular vacuoles. *Proteins that are normally in the glomerular filtrate are taken up by the cell membrane by pinocytosis*. Such pinocytotic vesicles fuse with primary lysosomes, which contain lytic enzymes. Secondary lysosomes are formed, and the macromolecular material is degraded or broken down. The products of low relative molecular mass then leave the lysosomes in order to prevent an increase in osmolality and lysosome swelling.

Potential nephrotoxins that may be taken into renal tubular cells in this manner include chelating agents such as nitrilotriacetic acid, ethylenediaminetetraacetic acid (EDTA), and metallothionein. Membrane binding of EDTA administered as the calcium-EDTA chelate persists, the calcium but not the EDTA being

dislocated to other cellular components. This suggests the way EDTA may sequester cellular lead or other metals for excretion.

G. Conclusion

The discovery and characterization of genetically programmed modes of cell death and their associated signaling networks have opened new avenues for studies of the mechanisms by which foreign chemicals might perturb cell functions and cause tissue damage. A host of novel targets have been identified, which could be affected either directly or indirectly by toxicants. Of particular significance is the critical role of Ca2+ and ROS in the modulation of several cell death modalities because a wide variety of toxic chemicals are known to affect cellular Ca2+ signaling and/or redox balance. *Hence, it appears that oxidative stress is a predominant mechanism of toxicity of the rapidly expanding group of engineered nanomaterials, although they might also interfere more directly with cell signaling.* The same is true for low doses, or mixtures of the more traditional environmental pollutants, which have been found to trigger apoptotic cell death in various in vitro models, although it remains questionable whether the same mechanisms are responsible for their *in vivo* toxicity. *Arsenic compounds are good examples of dose-dependent toxicity in view of their ability to trigger either apoptosis or autophagy by activation of the appropriate signaling network at low doses, whereas they kill cells by mitochondria-mediated necrosis at high doses.* There are several other examples of interference with physiological cell signaling by foreign chemicals, and more will probably follow. Hence, it is important to continue the work to unravel remaining secrets of cell death signaling in order to further our understanding of toxicity mechanisms and improve risk assessment.

In spite of the recent advances in our understanding of cell death mechanisms and associated signaling networks, much work remains to be done before we can fully appreciate the toxicological significance of these findings. Although the activation of cell death pathways is responsible for acute toxicity of many drugs and chemical toxicants, their potential involvement in sub-acute or chronic toxicity caused by long-term exposure to other drugs or environmental pollutants remains to be investigated. *An important question is then how we can translate observations from various in vitro models to in vivo toxicology*? *How can we compensate for differences in dose, tissue distribution, time of exposure, and the complexity of the biological system?* It is obvious that answers to these and similar questions require comparative studies under more *in vivo*–like conditions. However, the availability of novel technology and a host of suitably tailored biological models will hopefully help us overcome these difficulties and better understand the significance of cell death mechanisms for *in vivo* toxicology.

Questions

1. *Define mechanistic toxicology?*
2. *Significance of Route of Exposure in mechanistic toxicology?*
3. *Discuss on Impact of Pharmacokinetics on Toxicity?*
4. *What are the types of mechanisms of toxicity?*
5. *What are the Factors that modify cellular injury by toxins?*

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