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Nephrotoxicants and Nephrotoxicity Testing: An Outline Of *In Vitro* Alternatives

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Abstract

Exposure to drugs and chemicals often results in toxicity to living organisms. Drugs and chemicals are a common source of kidney injury. Compared with 30 years ago, the average patient today is older, has more comorbidity, and is exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function. Therefore, successful prevention requires knowledge of pathogenic mechanisms of renal injury, patient related risk factors, drug-related risk factors, and rapid screening procedures by which we can access the potential agents with nephrotoxicity. *In vitro* methods have been invaluable in helping to understand the mechanisms of well-established nephrotoxins. *In vitro* methods offer a rapid and economical method of screening specific cell types for specific effects. This insight has also been used to help screen new chemicals for their potential nephrotoxicity. *In vitro* technologies are advancing rapidly, improving the scientific validity of this approach, and extending their use. The future is therefore one in which more *in vitro* techniques will be used, better to answer questions regarding how to understand disease and improve health for animals and humans.

Key words: *Nephrotoxicants, Kidney injury, In vitro techniques, Economical*

INTRODUCTION

Exposure to drugs and chemicals often results in toxicity to living organisms. We must recognize the fact that not all compounds are equally toxic to all parts of a living system because the toxic actions of many compounds are manifested in specific organs. These organs are known as target organs of toxicity. [1].Drugs /chemicals are eliminated primarily by the kidney, lung, and/or liver. Consequently, these organs are frequently targets for toxicity produced by a variety of chemicals. The kidney may be much more susceptible than other organs to the toxic effects of a variety of chemicals for a number of reasons. The kidneys comprise only 0.4% of the body weight in most mammals, but receive 20-25% of the cardiac output, which ensures a high level of Xenobiotic delivery over a period of time, especially to the renal cortex which receives over 90% of the renal blood flow." Furthermore, the ability of the kidney to concentrate tubular fluid may enhance toxicity due to increased xenobiotic concentrations. Additionally, specialized functions of the proximal tubular cells may enhance toxicity in one or more of the following ways, all of which contribute to high intracellular concentrations of potentially toxic xenobiotics. Among them are Solutes reabsorbed by passive or active mechanisms pass through the tubular cells and direct secretion of many organic compounds into the tubular lumen by organic acid or base transport mechanisms, hence passing through or accumulating within the proximal tubular cells, exposing those cells to very high concentrations. Concentrating capability of the tubule produces high concentrations in the medullary lumen and interstitium. A large biotransformation capacity results in High metabolic rates and workload increases the sensitivity to toxicants. Metabolic alteration (biotransformation enzymes) may produce highly toxic metabolites or reactive intermediates. Additionally kidneys are Sensitive to vasoactive compounds and have large luminal membrane surface area, and Baseline medullary hypoxia. Even Metabolic alteration (biotransformation enzymes) may produce highly toxic metabolites or reactive intermediates which can serve as nephrotoxicants. [2], [3].

A vast number of nephrotoxicants can produce a variety of clinical syndromes-acute renal failure, chronic renal failure, nephrotic syndrome, hypertension and renal tubular defects. The evolving understanding of the toxicant-mediated renal injury has implications for potential therapies and preventive measures. This paper outlines list of nephrotoxicants, associated risk factors for nephrotoxicity, and various currently available invitro screening procedures for nephrotoxicants.

CLINICAL SIGNIFICANCE OF NEPHROTOXICANTS

- Nephrotoxins may account for approximately 50% of all cases of acute and chronic renal failure. Nephrotoxic renal injury often occurs in conjunction with ischemic acute renal failure.
- Acute renal failure may occur in 2% to 5% of hospitalized patients and 10% to 15% of patients in intensive care units.
- The mortality of acute renal failure is approximately 50% which has not changed significantly in the last 40 years.
- Radiocontrast media and aminoglycosides are the most common agents associated with nephrotoxic injury in hospitalized patients.
- Aminoglycoside nephrotoxicity occurs in 5% to 15% of patients treated with these drugs.
- Among older adults, the incidence of drug-induced nephrotoxicity may be as high as 66 percent.
- Compared with 30 years ago, patients today are older, have a higher incidence of diabetes and cardiovascular disease, take multiple medications, and are exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function. [4], [5], [6], [7], [8], [9], [10].

RISK FACTORS FOR NEPHROTOXICITY

Patient-related factors

- Age, sex, race
- Pre-existent renal disease
- Specific disease (diabetes mellitus, multiple myeloma, proteinuric patients, lupus)
- Sodium-retaining states (cirrhosis, heart failure, nephrosis)
- Dehydration and volume depletion
- Acidosis, potassium and magnesium depletion
- Hyperuricemia, hyperuricosuria
- Sepsis, shock
- Renal transplantation

Drug or chemical-related factors

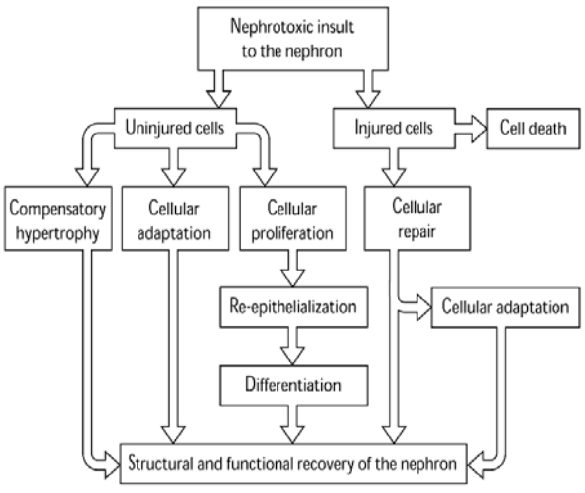
- Inherent nephrotoxic potential
- Dose
- Duration, frequency and form of administration
- Repeated exposure

Drug interactions

Combined or closely associated use of diagnostic or therapeutic with added or synergistic nephrotoxic potential (eg. Radiocontrast agents, aminoglycosides, NSAIDs, cisplatin, ACEI) [11]

Renal Cellular Responses to Nephrotoxicant Exposures

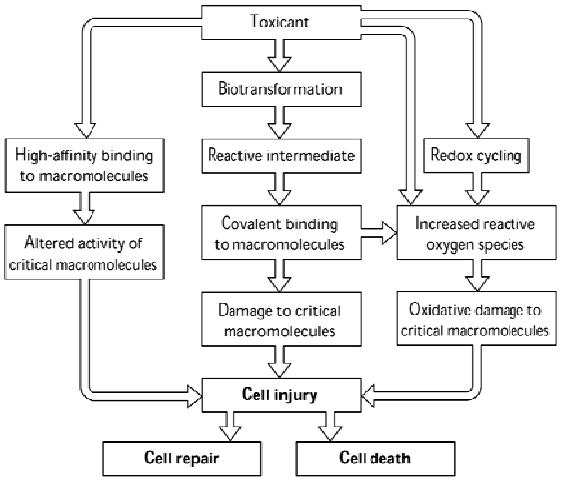
The nephron's response to a nephrotoxic insult. After populations of cells are exposed to a nephrotoxicant, the cells respond and ultimately the nephron recovers function or, if cell death and loss is extensive, nephron function ceases. Terminally injured cells undergo cell death through oncosis or apoptosis.



Cells injured sublethally undergo repair and adaptation (*eg*, stress response) in response to the nephrotoxicant. Cells not injured and adjacent to the injured area may undergo dedifferentiation, proliferation, migration or spreading, and differentiation. Cells that were not injured may also undergo compensatory hypertrophy in response to the cell loss and injury. Finally the uninjured cells may also undergo adaptation in response to nephrotoxicant exposure.

Mechanisms of Nephrotoxicant -Mediated Cellular Injury

Covalent and noncovalent binding versus oxidative stress mechanisms of cell injury. Nephrotoxicants are generally thought to produce cell injury and death through one of two mechanisms, either alone or in combination. In some cases the toxicant may have a high affinity for a specific macromolecule or class of macromolecules that result in altered activity (increase or decrease) of these molecules, resulting in cell injury. Alternatively, the parent nephrotoxicants may not be toxic until it is biotransformed into a reactive intermediate that binds covalently to macromolecules and in turn alters their activity, resulting in cell injury. Finally, the toxicant may increase reactive oxygen species in the cells directly, after being biotransformed into a reactive intermediate or through redox cycling. The resulting increase in reactive oxygen species results in oxidative damage and cell injury. [12], [13], [14]



SCREENING OF NEPHROTOXICANTS *IN-VITRO*

The kidney is a complex organ composed of over 20 different cell types, each with diverse morphological, biochemical and functional heterogeneity, but they work in concert to maintain normal renal function. [15], [16] It is this heterogeneity that provides a basis for renal injury and also ensures that *in vitro* methods offer the key to understanding these processes. Many nephrotoxicants (Table 1.) produce distinct patterns of injury localized to discrete cell types.

The Choice of *In Vitro* System

In vitro methods offer a rapid and economical method of screening specific cell types for specific effects. They offer systems in which the direct effects of chemicals can be evaluated and manipulated under precisely controlled conditions, in order to distinguish direct and indirect effects at a cellular and subcellular level. *In vitro* methods have been invaluable in helping to understand the mechanisms of well-established nephrotoxins. This insight has also been used to help screen new chemicals for their potential nephrotoxicity,

There are a number of considerations that need to be addressed before designing and interpreting an *in vitro* experiment for studying nephrotoxicity of chemicals/drugs, including:

1. Which of the different systems provides the most reliable data?
2. Which system really represents the renal tissue of interest?
3. What concentration of chemical should be used and how this can be related to the *in vivo* situation in animals and humans?
4. What end-point is most appropriate?
5. How does this relate to the situation *in vivo* and how relevant it is to pathological injury?
6. What are the advantages of using human renal tissue as the source of material for investigations?

Table 1: Groups of Nephrotoxic compounds to which humans are exposed

Antibiotics: Aminoglycosides (Gentamicin, Tobramycin, Amikacin, Netilmicin), Amphotericin B ,Cephalosporins, Ciprofloxacin, Demeclocycline, Penicillins, Pentamidine, Polymixins, Rifampin, Sulfonamides, Tetracycline, Vancomycin, Quinolones, Vancomycin, amphotericin B	**Other drugs** Acetaminophen,Halothane,Methoxyflurane, Cimetidine, Hydralazine, Lithium, Lovastatin, Mannitol,Penicillamine, Procainamide, Thiazides, Lindane, Clopidogrel , Ticlopidine, Amitriptyline, doxepin	**Organic Solvents And Chemicals** Ethylene Glycol, Carbon Tetrachloride,Unleaded Gasoline, Bipyridyl Herbicides Potassium Dichromate, D-Serine, Hexachloro 1,3 Butadiene, Chloroform
Chemotherapeutic agents Adriamycin Cisplatin,carboplatin Methotraxate Doxorubicin Mitomycin C, sulfonamides, Nitrosoureas (*eg*, streptozotocin, Iomustine)	**Plant toxins** Chinese herbals with aristocholic acid **Antihistamines** Diphenhydramine, doxylamine **Mycotoxins** Aflatoxins, Ochratoxin A Citrinin	**Radiocontrast media** **Ionic** (*eg*, diatrizoate, iothalamate) Nonionic (*eg*, metrizamide) **Recombinant peptides** Interferon-alfa **Immunosuppressive agents** Cyclosporin A, Tacrolimus
Antiviral agents Acyclovir, Cidovir, Foscarnet Valacyclovir, Ganciclovir,Foscarnet **Angiotensin receptor antagonists** Losartan	**Endogenous compouns** Myoglobin, Hemoglobin, Calcium,Uric acid, Oxalate, Cystine	**Drugs of abuse** Cocaine, heroin, ketamine, methadone, methamphetamine **Proton pump inhibitors** Lansoprazole, omeprazole , pantoprazole
Angiotensin-converting enzyme inhibitors: Captopril**,** Enalopril Lisinopril	**Heavy Metals** Cadmium Gold, Mercury, Lead ,Arsenic, Bismuth, Uranium,Chromium Germanium	**Nonsteroidal anti-inflammatory drugs (NSAIDs):**Ibuprofen, Naproxen,Indomethacin,Meclofenamate,Aspirin,Piroxicam

Table 2: Different approaches to investigate nephrotoxicity *in vitro*

Technique for assessing nephrotoxicity	***Advantages and limitations***
Anatomical relationship between cells maintained	
Perfusion, micropuncture	Technically difficult, requires sophisticated equipment, subject to artefact in inexperienced hands and difficult to interpret
Slices	Technically easier, no sophisticated equipment, less artefact and easier to interpret
Glomeruli and tubular fragments	Technically easy, some sophisticated equipment subject to artefact and easy to interpret
Anatomical relationship between cells lost	
Freshly isolated cells	Dispersal may damage cells and make it difficult to establish their anatomical identity unless there are clearly defined histochemical and immunocytochemical markers. Isolated cells are generally mixtures but may be enriched. Must be used within a few hours
Primary cell cultures	De-differentiate rapidly or change characteristics which may obfuscate their anatomical origins. Loss of a key biochemical characteristic may invalidate *in vitro* studies or alter sensitivity and selectivity
Established renal cell lines	Properties reminiscent of specific parts of the nephron. Often heterogeneous. Need to be characterized more systematically
Cell-free systems Vesicles, nuclei, lysosomes and microsomes	Study subcellular distribution, interactions between cellular compartments and a chemical, and the kinetics of binding or release of substances. Enzyme inhibition, metabolic activation, covalent binding and modulation of lipid peroxidation using purified or commercially available chemicals with appropriate cofactors

Different approaches to investigate nephrotoxicity *in vitro* [17-30]

A variety of *in vitro* techniques are available and include those where anatomical integrity is well maintained (perfusion, micropuncture and slices) and simple models such as isolated fragments (glomeruli and tubular fragments), cultures primary and continuous lines, and cell-flee systems These *in vitro* systems have helped understand the physiological processes in renal cells; each has strengths and weaknesses and some are technically difficult. (Table 2).

Some of the key considerations that have to be addressed when undertaking *in vitro* investigations on nephrotoxicity.

- Identification of compounds with well-documented *in vivo* nephrotoxicity, sequence of pathological functional changes, metabolites formed, quantities excreted and cellular pharmacodynamic effects
- Chemicals that target specifically for one anatomically discrete cell type *in vivo*
- Use analogues for structure-activity relationship
- Systematic study of compounds by several different *in vitro* methods
- Use of several criteria for assessing *in vitro* nephrotoxicity for each system [17-30]

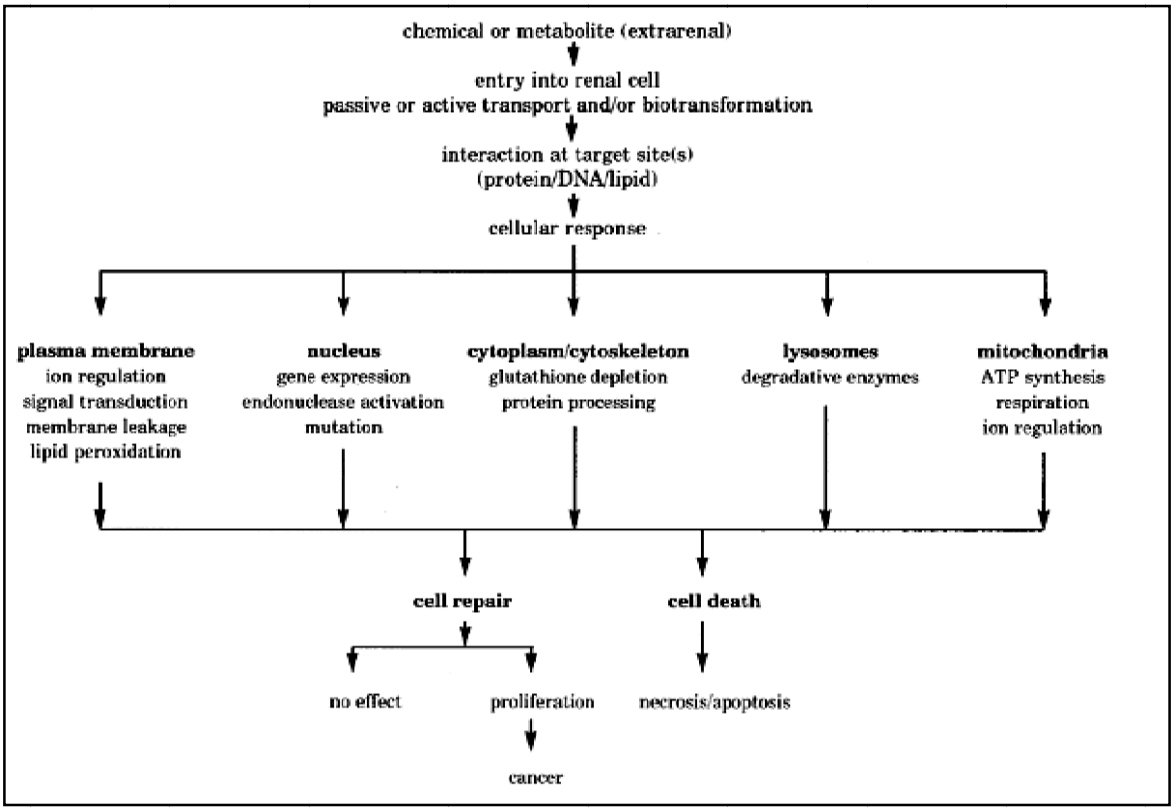
End points of Toxicity

There are a number of end-points depending on the uptake (such as anion, cation, glucose or chemical) and release (e.g. enzyme, anion, cation, chemical). In addition, the distribution of molecules, energy metabolism, and the synthesis, expression and turnover of specific markers, as well as light and ultrastructural morphology, has been applied to each of the *in vitro* techniques. End point may be of Morphological, Functional and Biochemical type.

Table 3

Endpoints	Applicability	Comments
Cell viability		
Dye exclusion/retention Enzyme/ion leakage	Renal cortical slices Renal fragments Isolated cells (in suspension) Primary cell cultures Cell lines	Applicable in intact cell systems and slices (for example K^+ content) Enzyme leakage provides information on regiospecificity NB: MTT assay is not specific for mitochondrial damage
Synthesis of macromolecules		
Protein DNA/RNA Matrix elements	Renal cortical slices Renal fragments Isolated cells Primary cell cultures Cell lines May be useful in glomerular/ interstitial cell cultures	Check uptake of precursors
Rate of proliferation		
(Clonogenic assays)	Cell lines	Total protein measurements (usually reflect cell number)
Carrier-mediated transport		
Glucose Organic ions Inorganic ions Low molecular weight proteins	Systems containing proximal tubule cells Isolated tubules (transepithelial transport) Cultures on porous membranes	Can be quantified by uptake or transport Uptake applicable to all transepithelial systems Check effects of specific inhibitors Check paracellular transport Not for routine use Not widely used
Endocytosis of labelled proteins or carbohydrates		
Cultured cells of proximal tubular origin		Usually use radiolabel or horse radish peroxidase Easy *in vivo/in vitro* comparison
Barrier function	All epithelial systems, intact epithelia or confluent cultures on porous membranes	Assessment of diffusion of extracellular markers, or electrophysiological measurements

Table 4



Morphological

Light microscopy is widely used for assessing cellular changes. The perfused kidney, renal tissue slices and tissue/cellular fragments can be fixed and processed by routine histopathology techniques, and can be assessed subjectively or quantitatively. The endpoints that are commonly used include lethal (necrosis) and sublethal injury, and the loss of accumulation of those cellular structures, macromolecules and /or activities which can be detected histochemically.

Functional End points

Various functional end points and their applicabilities in the invitro systems are summarized in Table-3

Biochemical End points

Various Biochemical end points and their applicabilities in the invitro systems are summarized in Table-4

FUTURE OUTLOOK

The kidney may be much more susceptible than other organs to the toxic effects of a variety of chemicals for a number of reasons. *In vitro* methods offer a rapid and economical method of screening specific cell types for specific effects. They offer systems in which the direct effects of chemicals can be evaluated and manipulated under precisely controlled conditions, in order to distinguish direct and indirect effects at a cellular and subcellular level. *In vitro* methods have been invaluable in helping to understand the mechanisms of well-established nephrotoxins. This insight has also been used to help screen new chemicals for their potential nephrotoxicity. *In vitro* technologies are advancing rapidly, improving the scientific validity of this approach, and extending their use. The future is therefore one in which more *in vitro* techniques will be used, better to answer questions regarding how to understand disease and improve health for animals and humans.

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