AOP ID and Title:

SNAPSHOT

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AOP 258: Renal protein alkylation leading to kidney toxicity

Short Title: Renal protein alkylation leading to kidney toxicity

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Abstract

It is well established that bioactivation of xenobiotics to reactive intermediates that covalently bind to proteins presents a major mechanism by which xenobiotics may cause proximal tubule injury. Examples for compounds that form covalent protein adducts in proximal tubule cells include haloalkenes (e.g. trichloroethylene, tetrachloroethylene, hexachloro-1,3-butadiene, chloroform), quinones (derived from e.g. hydroquinone, bromobenzene, 4-aminophenol), cephalosporins, and N-(3,5-dichlorophenyl)succinimide [1-6]. Covalent interaction of a chemical or a metabolite with cellular proteins represents the molecular initiating event (MIE) that triggers perturbation of cellular functions, of which mitochondrial dysfunction (KE1) leading to ATP depletion (KE2) appears to be most critical for proximal tubule cell death (KE3) by apoptosis and/or necrosis [5, 7-10]. Tubular obstruction and inflammatory responses to proximal tubule injury including activation of complement may cause secondary toxicity and thus amplify kidney injury, resulting in a progressive decline in kidney function (evidenced by e.g. rise in serum creatinine and blood urea nitrogen) (AO).

Summary of the AOP

Molecular Initiating Event

Title	Short name
Alkylation, Protein (https://aopwiki.org/events/244)	Alkylation, Protein

244: Alkylation, Protein (https://aopwiki.org/events/244)

Short Name: Alkylation, Protein

Key Event Component

Process	Object	Action
protein alkylation		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
38: Protein Alkylation leading to Liver Fibrosis (https://aopwiki.org/aops/38)	MolecularInitiatingEvent
258: Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	MolecularInitiatingEvent

Stressors

Name
Allyl Alcohol
Carbon tetrachloride
Retinol
Dimethyl nitrosamine
Thioacetamide

Biological Organization

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

Two prototypical chemicals acting via protein alkylation are Allyl Alcohol [12][13][6][14][15] and Carbon Tetrachloride (CCl4)[11][16] [17] [18] [19][20][21][22] . [23] [24] .[25] [26]

Covalent protein alkylation is a feature of many cytotoxic drugs but the overall extent of binding does not adequately distinguish toxic from non-toxic binding. [27] Interestingly, some chemicals significantly alkylate proteins without causing toxicity, which suggests that only alkylation of a specific protein subset critical subset contributes to injury. Indeed, Codreanu presented an inventory of proteins affected by electrophile-mediated alkylation in intact cells and suggested that non-toxic covalent binding largely affects cytoskeletal protein components, whereas toxic covalent binding induces lethal injury by targeting factors involved in protein synthesis and catabolism and possibly mitochondrial electron transport. [3] In vitro covalent binding studies to macromolecules have been used to elucidate the biochemical mechanisms of chemical-induced toxicity. Experimental work with kidney epithelial cells by Chen et al suggested that following alkylation of cellular macromolecules as initial cytotoxic event both sulfhydryl depletion and lipid peroxidation are components of the cytotoxic mechanism [28] Dennehy et al have analyzed the protein targets in nuclear and cytoplasmic proteomes from human embryonic kidney cells (HEK293) treated in vitro with two biotin-tagged, thiol-reactive electrophiles and mapped the adducts. Certain protein families appeared particularly susceptible to alkylation. [29] Shin et al have identified protein targets of two biotin-tagged model electrophiles in human liver microsomes through LC-MS-MS and showed that different target selectivities of the two electrophile probes correlated with different biological outcomes and that alkylation reactions of specific targets could be quantified. [30]

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Term	Scientific Term	Evidence	Links
Rattus norvegicus	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Human, rat and mouse [11]

How this Key Event Works

Alkylation is the transfer of an alkyl group from one molecule to another. The alkyl group may be transferred as an alkyl carbocation, a free radical, a carbanion or a carbene (or their equivalents). Protein alkylation is the addition of an alkyl group to a protein amino acid. An alkyl group is any group derived from an alkane by removal of one hydrogen atom. Alkylating agents are highly reactive chemicals that introduce alkyl groups into biologically active molecules and thereby prevent their proper functioning. Alkylating agents are classified according to their nucleophilic or electrophilic character. Nucleophilic alkylating agents deliver the equivalent of an alkyl anion (carbanion). These compounds typically can add to an electron-deficient carbon atom such as at a carbonyl group. Electrophilic alkylating agents deliver the equivalent of an alkyl cation. Alkyl halides can also react directly with amines to form C-N bonds; the same holds true for other nucleophiles such as alcohols, carboxylic acids, thiols, etc. Alkylation with only one carbon is termed methylation. [1] [2]

Covalent protein alkylation by reactive electrophiles was identified as a key triggering event in chemical toxicity over 40 years ago and these reactions remain a major cause of chemical-induced toxicity. Interestingly, some chemical molecules produce significant protein covalent binding without causing toxicity, which suggests that only a critical subset of protein alkylation events contributes to injury. The study by Codreanu et al. (2014) describes an inventory of electrophile- mediated protein damage in intact cells and suggests that non-toxic covalent binding may largely be survivable damage to cytoskeletal components, whereas toxic covalent binding produces lethal injury by targeting protein synthesis and catabolism and possibly mitochondrial electron transport. [3] [4] [5] [6] [7]

How it is Measured or Detected

HPLC-ESI-MS/MS analysis

High Performance Liquid Chromatography – electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) is the most popular MS technique. It combines the separation ability of HPLC along with the sensitivity and specificity of detection from MS. One of the advantages of HPLC-MS is that it allows samples to be rapidly desalted online, so no sample preparation is required unlike samples for GC-MS. Electrospray ionisation can produce singly or multiply charged ions. Typically high molecular weight compounds have multiple charges i.e. peptides and proteins. This technique is particularly suited to analysing polar molecules of mass <2000 Dalton and requires no prior derivatisation in most applications. [8] [3] [9]

MALDI-TOF/MS (Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry)

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (biopolymers such as DNA, proteins, peptides and sugars) and large organic molecules (such as polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods. MALDI methodology is a three-step process. First, the sample is mixed with a suitable matrix material and applied to a metal plate. Second, a pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material. Finally, the analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases, and can then be accelerated into whichever mass spectrometer is used to analyse them. [10]

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Key Events

Title	Short name
Dysfunction, Mitochondria (https://aopwiki.org/events/1483)	Dysfunction, Mitochondria
Decrease, Mitochondrial ATP production (https://aopwiki.org/events/40)	Decrease, Mitochondrial ATP production
Increase, Cytotoxicity (renal tubular cell) (https://aopwiki.org/events/709)	Increase, Cytotoxicity (renal tubular cell)

1483: Dysfunction, Mitochondria (https://aopwiki.org/events/1483)

Short Name: Dysfunction, Mitochondria

AOPs Including This Key Event

AOP ID and Name	Event Type
256: Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity (https://aopwiki.org/aops/256)	KeyEvent
258: Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	KeyEvent

Biological Organization

Level of Biological Organization
Cellular

40: Decrease, Mitochondrial ATP production (https://aopwiki.org/events/40)

Short Name: Decrease, Mitochondrial ATP production

Key Event Component

Process	Object	Action
ATP biosynthetic process	ATP	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
26: Calcium-mediated neuronal ROS production and energy imbalance (https://aopwiki.org/aops/26)	KeyEvent
238: Excessive reactive oxygen species production leading to reproductive failure (https://aopwiki.org/aops/238)	KeyEvent
245: Uncoupling of photophosphorylation leading to reduced ATP production associated growth inhibition (https://aopwiki.org/aops/245)	KeyEvent
258: Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	KeyEvent

Biological Organization

Level of Biological Organization
Cellular

Cell term

Cell term
eukaryotic cell

709: Increase, Cytotoxicity (renal tubular cell) (https://aopwiki.org/events/709)

Short Name: Increase, Cytotoxicity (renal tubular cell)

Key Event Component

Process	Object	Action
cell death	kidney tubule cell	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
105: Alpha2u-microglobulin cytotoxicity leading to renal tubular adenomas and carcinomas (in male rat) (https://aopwiki.org/aops/105)	KeyEvent
256: Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity (https://aopwiki.org/aops/256)	KeyEvent
257: Receptor mediated endocytosis and lysosomal overload leading to kidney toxicity (https://aopwiki.org/aops/257)	KeyEvent
258: Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	KeyEvent

Biological Organization

Level of Biological Organization
Cellular

Cell term

Cell term
kidney tubule cell

Adverse Outcomes

Title	Short name
Occurrence, Kidney toxicity (https://aopwiki.org/events/814)	Occurrence, Kidney toxicity

814: Occurrence, Kidney toxicity (https://aopwiki.org/events/814)

Short Name: Occurrence, Kidney toxicity

Key Event Component

Process	Object	Action
toxicity	kidney	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
128: Kidney dysfunction by decreased thyroid hormone (https://aopwiki.org/aops/128)	AdverseOutcome
256: Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity (https://aopwiki.org/aops/256)	AdverseOutcome
257: Receptor mediated endocytosis and lysosomal overload leading to kidney toxicity (https://aopwiki.org/aops/257)	AdverseOutcome
258: Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	AdverseOutcome

Biological Organization

Level of Biological Organization
Organ

Organ term

Organ term
kidney

Scientific evidence supporting the linkages in the AOP

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Alkylation, Protein	directly leads to	Dysfunction, Mitochondria	Not Specified	Weak

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Dysfunction, Mitochondria	directly leads to	Decrease, Mitochondrial ATP production	Strong	Weak
Decrease, Mitochondrial ATP production	directly leads to	Increase, Cytotoxicity (renal tubular cell)	Strong	Weak
Increase, Cytotoxicity (renal tubular cell)	directly leads to	Occurrence, Kidney toxicity	Strong	Moderate

Alkylation, Protein leads to Dysfunction, Mitochondria (https://aopwiki.org/relationships/1680)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	**directly leads to**	**Not Specified**	**Weak**

Dysfunction, Mitochondria leads to Decrease, Mitochondrial ATP production (https://aopwiki.org/relationships/1681)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	**directly leads to**	**Strong**	**Weak**

Decrease, Mitochondrial ATP production leads to Increase, Cytotoxicity (renal tubular cell) (https://aopwiki.org/relationships/1682)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	**directly leads to**	**Strong**	**Weak**

Increase, Cytotoxicity (renal tubular cell) leads to Occurrence, Kidney toxicity (https://aopwiki.org/relationships/1676)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of mitochondrial DNA polymerase gamma leading to kidney toxicity (https://aopwiki.org/aops/256)	**directly leads to**	**Strong**	**Moderate**
Receptor mediated endocytosis and lysosomal overload leading to kidney toxicity (https://aopwiki.org/aops/257)	**directly leads to**	**Strong**	**Moderate**
Renal protein alkylation leading to kidney toxicity (https://aopwiki.org/aops/258)	**directly leads to**	**Strong**	**Moderate**

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Human, rat, mouse	Human, rat, mouse	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

Sex Applicability

Sex	Evidence
Unspecific	Strong

Weight of Evidence Summary

Concordance of dose-response relationships

This is still a qualitiative description of the pathway. There is at present no quantitative information on dose-response relationships. Experiments are underway to provide quantitative understanding of dose-response relationships and response-response relationships between upstream and downstream KEs.

Temporal concordance among the key events and adverse outcome

The individual KEs are shown to occur prior to or concomitant with the onset of nephrotoxicity.

Strength, consistency, and specificity of association of adverse outcome and initiating event

The scientific evidence on the association between protein alkylation by reactive intermediates and kidney toxicity (AO) is strong and consistent. The MIE is not specific for kidney toxicity and is well established to lead to damage to other organs, whereby the site of toxicity is largely determined by the toxicokinetics of the parent compound or active metabolite.

Biological plausibility, coherence, and consistency of the experimental evidence

The described AOP is biologically plausible, coherent and well supported by experimental data.

Alternative mechanism(s) that logically present themselves and the extent to which they may distract from the postulated AOP

There are no alternative mechanism(s) that logically present themselves, although a contribution of other mechanisms such as generation of oxidative stress to the overall AO is possible.

Uncertainties, inconsistencies and data gaps

This AOP is plausible and consistent with general biological knowledge. However, there is currently little understanding as to which target proteins are critical to toxicity mediated by alkalation damage. Quantitative information on dose response-relationships as well as response-response relationships for upstream and downstream KEs is needed to support its applicability for the development of alternative in vitro tests for nephrotoxicity testing.

Quantitative Consideration

Quantitative data on KERs between upstream and downstream KE are still lacking.

Considerations for Potential Applications of the AOP (optional)

The described AOP is intended to provide a mechanistic framework for the development of in vitro bioactivity assays capable of predicting quantitative points of departure for safety assessment with regard to nephrotoxicity. Such assays may form part of an integrated testing strategy to reduce the need for repeated dose toxicity studies (e.g. OECD Guideline 407; OECD Guideline 407).

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