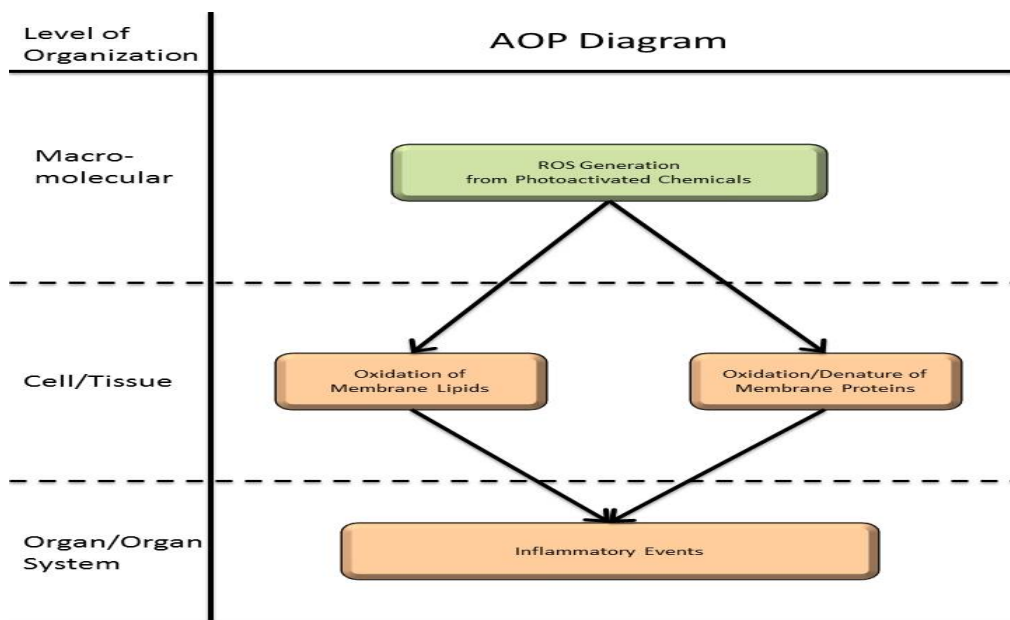


AOP 282: Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions

Short Title: ROS-mediated chemical phototoxicity

Graphical Representation



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Abstract

Phototoxicity is an adverse reaction in the light-exposed tissues triggered by normally harmless doses of sunlight (Moore, 1998; Moore, 2002; Roberts, 2001). Recently, high-intensity UV rays from the sun have reached the Earth's surface with the destruction of the ozone layer, and interest in phototoxic events has increased enormously. Notably, phototoxic reactions against exogenous agents are caused by the combined effects of environmental light and external agents, including drugs, cosmetics, and foods (Epstein, 1983; Stein and Scheinfeld, 2007).

In this AOP, the primary trigger for a compound to be considered with respect to potential to create photochemical and photobiological reactions is the absorption of photon energy from light ranging from 290 to 700 nm. The extent of absorption depends on the wavelength of light and the type of absorbing chromophores in the light-exposed tissues. A molecule is excited by absorption of photon energy, and the photoactivated molecule

induces photochemical reactions via energy transfer (type I photochemical reaction) and free radical generation (type II photochemical reaction). These photochemical reactions result in generation of radicals and reactive oxygen species, and the reactive species react with biomolecules. Generated radicals of a target chemical bind to DNA and proteins, resulting in formation of these photo-adducts, and reactive oxygen species (ROS), including singlet oxygen and superoxide, induce oxidation of biomolecules. These key events bring inflammatory events in the light-exposed tissues (Brendler-Schwaab et al. , 2004; Epstein and Wintroub, 1985; Quintero and Miranda, 2000).

This AOP describes the pathway of photochemical toxicity between attack of ROS generated from photoactivated chemicals to membranes and inflammatory events in light-exposed tissues.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	1592	ROS generation from photoactivated chemicals (https://aopwiki.org/events/1592)	ROS generation
	KE	1594	Oxidation of membrane lipids (https://aopwiki.org/events/1594)	Oxidation of membrane lipids
	KE	1595	Oxidation/denaturation of membrane proteins (https://aopwiki.org/events/1595)	Oxidation/denaturation of membrane proteins
	AO	1599	Inflammatory events in light-exposed tissues (https://aopwiki.org/events/1599)	Inflammatory events

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
ROS generation from photoactivated chemicals (https://aopwiki.org/relationships/1845)	adjacent	Oxidation of membrane lipids	High	Low
ROS generation from photoactivated chemicals (https://aopwiki.org/relationships/1846)	adjacent	Oxidation/denaturation of membrane proteins	High	Low
Oxidation of membrane lipids (https://aopwiki.org/relationships/1850)	adjacent	Inflammatory events in light-exposed tissues	High	Low
Oxidation/denaturation of membrane proteins (https://aopwiki.org/relationships/1851)	adjacent	Inflammatory events in light-exposed tissues	High	Low

Stressors

Name	Evidence
Light (290-700 nm)	High
Photoreactive chemicals	High
Reactive oxygen species	High

Overall Assessment of the AOP

The focus of this AOP is on photochemical toxicity, especially photoactivation of target chemicals followed by generation of ROS. ROS generated from photoirradiated chemicals can react with molecules on the membranes, including lipids and proteins, and the reactions may lead to inflammatory events in the UV-exposed tissues.

Phototoxicity is an adverse reaction triggered by normally harmless doses of sunlight. There are two types of photosensitive disorders, endogenous and exogenous phototoxicity, and the observable changes to the sunlight-exposed tissues are essentially detrimental, and include the following appearance; (i) immediate faint erythema during exposure, (ii) delayed erythematous responses, (iii) abnormal keratinisation and vacuolated cells, (iv) formation of desquamating layer, and (v) desquamation (peeling) (Moore, 1998; Moore, 2002; Roberts, 2001). Recently, high-intensity UV rays from the sun have reached the Earth's surface with the destruction of the ozone layer, and interest in phototoxic events has increased enormously. Notably, phototoxic reactions against exogenous agents are caused by the combined effects of UV irradiation and external agents, including drugs, cosmetics and foods (Stein and Scheinfeld, 2007). Phototoxic skin responses after administration of photosensitive drugs, so-called drug-induced phototoxicity, have been recognized as undesirable side effects, and several classes of drugs, even when not toxic by themselves, may become reactive under exposure to environmental light, inducing undesired phototoxic responses (Epstein, 1983).

The primary trigger for a compound to be considered with respect to potential to create photochemical and photobiological reaction is the absorption of UV and visible light ranging from 290 to 700 nm. The extent of absorption depends on the wavelength of light and the type of absorbing chromophores in the UV-exposed tissues. UV radiation is usually divided into several ranges based on its physiologic effects: (1) UVA (near UV): 320–400 nm (UVA I: 340–400 nm and UVA II: 320–340 nm), (2) UVB (middle UV): 290–320 nm, and (3) UVC (far UV): 180–290 nm (Svensson et al., 2001; Vassileva et al., 1998). The sun emits ultraviolet radiation in the UVA, UVB, and UVC bands, but because of absorption by the atmosphere's ozone layer, the main ultraviolet radiation that reaches the Earth's surface is UVA (Dubakiene and Kupriene, 2006). Absorption of light through the skin and eyes, primarily in the 290–700 nm range, varies with wavelength, such that light in the red region of the spectrum reaches well into the subcutis layer; whereas at 300 nm or shorter wavelength, only an estimated 10% passes through the epidermis (Epstein, 1989). Thus, penetration and absorption of light in the UV-exposed tissues is important factor in drug-induced phototoxicity as Grotthus-Draper law of photobiology states; only light that is absorbed can be active in photochemical and photobiological processes.

When a drug molecule absorbs a photon energy, electrons can be prompted from occupied orbitals (the ground state) to an unoccupied orbital (S1, S2) depending upon bond type and associated energy level. Furthermore, unpaired singlet state electrons (opposite spin) may be converted to triplet state (parallel spin) by inversion of the spin via intersystem crossing of the absorbed energy. To return to the ground state from S1, S2/T1, T2, energy must be dissipated by internal conversion, fluorescence (from singlet state), phosphorescence (from triplet state) or via chemical reaction, giving rise to photoproducts and/or potential external reactions with biomolecules.

In addition, molecular oxygen, a triplet radical in its ground state, appears to be the predominant acceptor of excitation energy as its lowest excited level (singlet state) has a comparatively low value. An energy transfer from excited triplet photosensitizer to the oxygen (type II photochemical reaction) could produce excited singlet oxygen which might, in turn, participate in a lipid- and protein-membrane oxidation or induce DNA damage. An electron or hydrogen transfer could lead to the formation of free radical species (type I photochemical reaction), producing a direct attack on the biomolecules or in the presence of oxygen, to evolve towards secondary free radicals such as peroxy radicals or the very reactive hydroxyl radical, a known intermediate in the oxidative damage of biomolecules. This toxic pathway corresponds to successive reactions which involve the appearance of superoxide anion radical, its dismutation to form hydrogen peroxide followed with the hydrogen peroxide reduction to form hydroxyl radical. Herein, excitation of the drug by light may give rise to ROS such as singlet oxygen and superoxide, which may be one of causative molecules for the drug-induced phototoxicity (Brendler-Schwaab et al., 2004; Epstein and Wintroub, 1985).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This AOP applies to a wide range of chemicals. Phototoxic chemicals are recognized to have following characteristics: (i) absorption of light within the range of natural sunlight (290-700 nm); (ii) generation of a reactive species following absorption of UV-visible light; (iii) distribution to light-exposed tissues (e.g., skin and eye) (ICH S10).

Sex: This AOP applies to both males and females.

Life stages: The relevant life stages for this AOP are all life stages after born.

Taxonomic: This AOP mainly applies to human.

Essentiality of the Key Events

The essentiality of KEs for this AOP was rated high on the basis of experimental evidence in the investigations related to each of KEs and published guidelines. For details see the table on "Support for Essentiality of KEs".

Weight of Evidence Summary

Support for biological plausibility of KERs

MIE => KE 1	Generated ROS from photoactivated chemicals can react with membrane lipids, and oxidation of membrane lipids could be occurred.	Biological Plausibility of the MIE => KE 1 is high. The relationship between MIE and KE 1 is consistent with chemical and biological knowledge (Girotti, 1990; Girotti, 2001; Onoue and Tsuda, 2006).
MIE => KE 2	Generated ROS from photoactivated chemicals can react with membrane proteins, and oxidation/denaturation of membrane proteins could be occurred.	Biological Plausibility of the MIE => KE 2 is high. The relationship between MIE and KE 2 is consistent with biological knowledge (Dalle Carbonare and Pathak, 1992; Valenzano, 1987).
KE 1 => AO	Oxidation of membrane lipids relates with damage produced in the cellular membrane, leading to inflammatory events.	Biological Plausibility of the KE 1 => AO is high. The relationship between KE 1 and AO is consistent with biological knowledge (Castell et al., 1994).
KE 2 => AO	Oxidation/denaturation of protein provides the necrosis of the living tissues as an inflammatory event.	Biological Plausibility of the KE 2 => AO is high. The relationship between KE 2 and AO is consistent with biological knowledge (Dalle Carbonare and Pathak, 1992; Opie, 1962).

Support for Essentiality of KEs

MIE	ROS generation from photoactivated chemicals	High; well-accepted generation of reactive oxygen species from photo-activated chemicals associated with phototoxic reactions with 200 of chemicals evaluated in qualitative endpoints (Onoue et al., 2014; Onoue et al., 2013a; Onoue et al., 2008a; Onoue and Tsuda, 2006; Seto et al., 2013b). The event has described in ICH S10 guideline as a crucial factor of phototoxic reactions (ICH, 2014).
KE 1	Oxidation of membrane lipids	High; Oxidative stress to lipids associated with the phototoxic reactions (Girotti, 1990; Girotti, 2001; Onoue and Tsuda, 2006).
KE 2	Oxidation/denaturation of membrane proteins	High; accepted oxidation/denaturation of proteins associated with the phototoxic reactions (Dalle Carbonare and Pathak, 1992; Valenzano, 1987).
Adverse outcome	Inflammatory events in sunlight-exposed tissues	Photoreactive agents indicated inflammatory events, including edema, dyskeratosis, and necrosis, in light-exposed tissues after sunlight exposure (Moore, 1998; Moore 2002; Roberts, 2001).

Empirical Support for KERs

MIE => KE 1: ROS generation leads to Oxidation of membrane lipids	<p>Empirical support of the MIE => KE 1 is strong.</p> <p>Rationale:</p> <p>Lipid peroxidation was occurred by ROS-generating chemicals under exposure to simulated sunlight (Onoue et al., 2011, Onoue and Tsuda, 2006).</p> <p>A photoreactive chemical indicated dose-dependent increases in ROS generation and lipid peroxidation after exposure to a fixed dose of simulated sunlight (Seto et al., 2013a).</p>
MIE => KE 2: ROS generation leads to Oxidation/denaturation of membrane proteins	<p>Empirical support of the MIE => KE 2 is moderate.</p> <p>Rationale:</p> <p>ROS generated from photosensitizing agents led to oxidation and denaturation of proteins (Dalle Carbonare and Pathak, 1992).</p>
KE 1 => AO: Oxidation of membrane lipids leads to Inflammatory events	<p>Empirical support of the KE 1 => AO is strong.</p> <p>Rationale:</p> <p>Increases in lipid peroxidation and inflammatory-related cytokines were observed in the murine skin, and naringenin, an anti-oxidant, attenuated these increases in a dose-dependent manner (Martinez et al., 2015).</p> <p>Benzoyl peroxide, a ROS generator, led to lipid peroxidation and GSH depletion, and the changes caused the gene expression of pro-inflammatory cytokines (Valacchi et al., 2001).</p>

KE 2 => AO: Oxidation/denaturation of membrane proteins leads to Inflammatory events	Empirical support of the KE 2=> AO is moderate. Rationale: Denaturation of proteins induced necrosis and inflammatory in the skin (Opie, 1962).
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Quantitative Consideration

Although there is empirical information on KERs as described above sections, the overall quantitative understanding of the AOP is insufficient to directly link a measure of KEs to a quantitative prediction of KERs.

As a pre-MIE, light absorption of chemicals is an important event for phototoxic reactions induced by photoreactive chemicals. Quantitative endpoint on absorption of light (290–700 nm) was recognized in the previous report (Henry et al., 2009), and, for photoreactive chemicals, the criterion on molar extinction coefficient (MEC) was determined to be $1,000 \text{ M}^{-1}\cdot\text{cm}^{-1}$. Most of chemicals with MEC values of over $1,000 \text{ M}^{-1}\cdot\text{cm}^{-1}$ generated significant ROS, including singlet oxygen and/or superoxide (Onoue et al., 2013b; Onoue and Tsuda, 2006), and the qualitative criteria on ROS generation was determined to evaluate chemical phototoxicity (Onoue et al., 2014; Onoue et al., 2013a; Onoue et al., 2008b).

Considerations for Potential Applications of the AOP (optional)

The MIE and KEs in this AOP could contribute to assays development for photosafety evaluation and an AOP-based IATA construction. AOP-based IATA can be applied for various aims including screening of chemicals, prioritization of chemicals for further testing, and risk assessment.

The regulatory applicability of the AOP would be to use experimental results from assays based on MIE and KEs as indicators for the risk of phototoxic reactions.

Combined use of photobiochemical properties and tissue exposure data would be of help for photosafety evaluation of chemicals. Risk assessment would be possible when exposure data in light-exposed tissues combined with assay data based on AOP.

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Appendix 1

List of MIEs in this AOP

Event: 1592: ROS generation from photoactivated chemicals (<https://aopwiki.org/events/1592>)

Short Name: ROS generation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	MolecularInitiatingEvent

Stressors

Name
Light (290-700 nm)
Photoreactive chemicals

Biological Context

Level of Biological Organization
Molecular

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

Several classes of chemicals cause ROS generation under light exposure, and the ROS generation can be monitored by ROS assay (Onoue et al., 2014, Onoue et al., 2013, Onoue et al., 2008, Seto et al., 2013). The criteria of ROS assay for photosafety assessment of chemicals were defined (Onoue et al., 2014, Onoue et al., 2013).

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This MIE applies to a wide range of chemicals. The chemicals absorb photon energy from light within the range of natural sunlight (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

Sex: This MIE applies to both males and females.

Life stages: The relevant life stages for this MIE are all life stages after born.

Taxonomic: This MIE mainly applies to human.

Key Event Description

In the primary event, photoreactive chemicals are excited by the absorption of photon energy. The energy of the photoactivated chemicals transfer to oxygen and then generates the reactive oxygen species (ROS), including superoxide (O_2^-) via type I reaction and singlet oxygen (1O_2) via type II reaction, as principal intermediate species in phototoxic reaction (Foote, 1991, Onoue et al., 2009).

How it is Measured or Detected

On the basis of the pathogenesis of drug-induced phototoxicity, a reactive oxygen species (ROS) assay was proposed to evaluate the phototoxic risk of chemicals. The ROS assay can monitor generation of ROS, such as singlet oxygen and superoxide, from photoirradiated chemicals, and the ROS data can be used to evaluate the photoreactivity of chemicals (Onoue et al., 2014, Onoue et al., 2013, Onoue and Tsuda, 2006). The ROS assay is a recommended approach by guidelines to evaluate the phototoxic risk of chemicals (ICH, 2014, PCPC, 2014).

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List of Key Events in the AOP

Event: 1594: Oxidation of membrane lipids (<https://aopwiki.org/events/1594>)

Short Name: Oxidation of membrane lipids

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	KeyEvent

Stressors

Name
Photoactivated chemicals
Reactive oxygen species

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This KE applies to a wide range of chemicals. The chemicals generate a reactive species, such as reactive oxygen species, following absorption of photon energy from light within the range of natural sunlight (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

Sex: This KE applies to both males and females.

Life stages: The relevant life stages for this KE are all life stages after born.

Taxonomic: This KE mainly applies to human.

Key Event Description

Lipid peroxidation of membrane lipids has been considered to be one of the major mechanisms in phototoxic skin responses induced by photoreactive chemicals (Castell et al., 1994, Girotti, 1990, 2001, Onoue et al., 2011, Onoue and Tsuda, 2006, Seto et al., 2013).

How it is Measured or Detected

An *in vitro* system using thiobarbituric acid can monitor lipid peroxidation by photoactivated chemicals (Onoue et al., 2011, Onoue and Tsuda, 2006).

References

Castell JV, Gomez-Lechon MJ, Grassa C, Martinez LA, Miranda MA, Tarrega P. Photodynamic lipid peroxidation by the photosensitizing nonsteroidal antiinflammatory drugs suprofen and tiaprofenic acid. *Photochem Photobiol.* 1994;59:35-9.

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Event: 1595: Oxidation/denatuation of membrane proteins (<https://aopwiki.org/events/1595>)

Short Name: Oxidation/denatuation of membrane proteins

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	KeyEvent

Stressors

Name
Photoactivated chemicals
Reactive oxygen species

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This KE applies to a wide range of chemicals. The chemicals generate a reactive species, such as reactive oxygen species, following absorption of photon energy from light within the range of natural sunlight (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

Sex: This KE applies to both males and females.

Life stages: The relevant life stages for this KE are all life stages after born.

Taxonomic: This KE mainly applies to human.

Key Event Description

Reactive oxygen species yielded from photoactivated chemicals can cause cross-linking of proteins and oxidation of sulfhydryl groups resulting in disulfide cross-links (Dalle Carbonare and Pathak, 1992).

How it is Measured or Detected

As for in vitro systems, sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and other gel electrophoresis methodologies can detect denaturation of proteins (Dalle Carbonare and Pathak, 1992).

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Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J Photochem Photobiol B. 1992;14:105-24.

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Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. Pharmaceutical research. 2006;23:156-64.

List of Adverse Outcomes in this AOP

Event: 1599: Inflammatory events in light-exposed tissues (<https://aopwiki.org/events/1599>)

Short Name: Inflammatory events

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:282 - Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	AdverseOutcome

Stressors

Name
Light (290-700 nm)
Photoreactive chemicals
Reactive oxygen species

Biological Context

Level of Biological Organization
Organ

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This AO applies to a wide range of chemicals. Phototoxic chemicals are recognized to have following characteristics: (i) absorption of light within the range of natural sunlight (290-700 nm); (ii) generation of a reactive species following absorption of UV-visible light; (iii) distribution to light-exposed tissues (e.g., skin and eye) in ICH S10 guideline for photosafety assessment (ICH, 2014).

Sex: This AO applies to both males and females.

Life stages: The relevant life stages for this AO are all life stages after born.

Taxonomic: This AO mainly applies to human.

Key Event Description

Photoirritation is frequently characterized as exaggerated sunburn sometimes mediated by oxidative stress in the cell membrane, and hyperpigmentation and desquamation may occur as a residual effect of a phototoxic reaction. Theoretically, if a high concentration of a phototoxic drug accumulates in the skin and the appropriate wavelength of light is present, any individual will develop a phototoxic reaction. In particular, peroxidation of membrane lipid could be induced by some photosensitizers under photo-irradiation, and this photochemical reaction certainly correlates with damage produced in the cell membrane, leading to the skin photoirritation (Castell et al. , 1994, Onoue et al. , 2009).

How it is Measured or Detected

Inflammatory events induced by photoreactive chemicals can be detected *in vivo* phototoxicity testing and photopatch test in clinical (Epstein, 1964, ICH, 2014, Onoue et al., 2009).

Regulatory Significance of the AO

Inflammatory events in light-exposed tissues are considered to be the endpoint of ROS-mediated chemical phototoxicity, especially photoirritant reactions (ICH, 2014, Onoue et al., 2009).

References

Castell JV, Gomez-Lechon MJ, Grassa C, Martinez LA, Miranda MA, Tarrega P. Photodynamic lipid peroxidation by the photosensitizing nonsteroidal antiinflammatory drugs suprofen and tiaprofenic acid. *Photochem Photobiol.* 1994;59:35-9.

Epstein S. The Photopatch Test; Its Technique, Manifestations, and Significance. *Ann Allergy.* 1964;22:1-11.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Seto Y, Gandy G, Yamada S. Drug-induced phototoxicity; an early *in vitro* identification of phototoxic potential of new drug entities in drug discovery and development. *Current drug safety.* 2009;4:123-36.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1845: ROS generation leads to Oxidation of membrane lipids (<https://aopwiki.org/relationships/1845>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This KER applies to a wide range of chemicals. The chemicals absorb photon energy from light within the range of light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

Sex: This KER applies to both males and females.

Life stages: The relevant life stages for this KER are all life stages after born.

Taxonomic: This KER mainly applies to human.

Key Event Relationship Description

Some photoactivated chemicals can generate reactive oxygen species (ROS) after photoactivation of chemicals by irradiation of light (290–700 nm). ROS generated from photoactivated chemicals can react with membrane lipids and lead to oxidation of membrane lipids.

Evidence Supporting this KER

Biological Plausibility

Photoactivated chemicals generate ROS, and the ROS-generating chemicals cause lipid peroxidation under exposure to light (290–700 nm) in chemical and biological systems (Girotti, 1990, 2001, Onoue and Tsuda, 2006).

Empirical Evidence

Lipid peroxidation was occurred by ROS-generating chemicals under exposure to simulated sunlight (Onoue et al. , 2011, Onoue and Tsuda, 2006).

A photoreactive chemical indicated dose-dependent increases in ROS generation and lipid peroxidation after exposure to a fixed dose of simulated sunlight (Seto et al. , 2013).

References

Girotti AW. Photodynamic lipid peroxidation in biological systems. Photochem Photobiol. 1990;51:497-509.

Girotti AW. Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. J Photochem Photobiol B. 2001;63:103-13.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Seto Y, Ochi M, Inoue R, Ito H, Hatano T, et al. In vitro photochemical and phototoxicological characterization of major constituents in St. John's Wort (*Hypericum perforatum*) extracts. *Phytochemistry*. 2011;72:1814-20.

Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. *Pharmaceutical research*. 2006;23:156-64.

Seto Y, Inoue R, Kato M, Yamada S, Onoue S. Photosafety assessments on pirfenidone: photochemical, photobiological, and pharmacokinetic characterization. *J Photochem Photobiol B*. 2013;120:44-51.

Relationship: 1846: ROS generation leads to Oxidation/denatuation of membrane proteins (<https://aopwiki.org/relationships/1846>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This KER applies to a wide range of chemicals. The chemicals absorb photon energy from light within the range of light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

Sex: This KER applies to both males and females.

Life stages: The relevant life stages for this KER are all life stages after born.

Taxonomic: This KER mainly applies to human.

Key Event Relationship Description

Some photoactivated chemicals can generate reactive oxygen species (ROS) after photoactivation of chemicals by irradiation of light (290–700 nm). ROS generated from photoactivated chemicals can react with membrane proteins and lead to oxidation/denaturation of membrane proteins.

Evidence Supporting this KER

Biological Plausibility

Photoactivated chemicals by UVA generate ROS including singlet oxygen, superoxide, and hydroxyl radicals, and the generated ROS cause cross-linking of proteins and denaturation of proteins as oxidative damages by photoactivated chemicals (Dalle Carbonare and Pathak, 1992).

Empirical Evidence

ROS generated from photosensitizing agents led to oxidation and denaturation of proteins, resulting in cross-linking of proteins and oxidation of sulfhydryl groups (Dalle Carbonare and Pathak, 1992).

References

Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. *J Photochem Photobiol B*. 1992;14:105-24.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical

Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. *Pharmaceutical research*. 2006;23:156-64.

Relationship: 1850: Oxidation of membrane lipids leads to Inflammatory events (<https://aopwiki.org/relationships/1850>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This KER applies to a wide range of chemicals. The chemicals generate ROS under exposure to light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

Sex: This KER applies to both males and females.

Life stages: The relevant life stages for this KER are all life stages after born.

Taxonomic: This KER mainly applies to human.

Key Event Relationship Description

Oxidation of membrane lipids can lead to inflammatory events via oxidative damage of cellular membranes and increases in expression of pro-inflammatory cytokines.

Evidence Supporting this KER

Biological Plausibility

Lipid peroxidation in cellular membrane is an oxidative damage of cellular membrane, and viability of cells decreases by lipid peroxidation of photoreactive chemicals (Castell et al., 1994).

Empirical Evidence

Increases in lipid peroxidation and inflammatory-related cytokines were observed in the murine skin, and naringenin, an anti-oxidant, attenuated these increases in a dose-dependent manner (Martinez et al., 2015).

Benzoyl peroxide, a ROS generator, led to lipid peroxidation and GSH depletion, and the changes caused the gene expression of pro-inflammatory cytokines in human keratinocytes (Valacchi et al., 2001).

References

Castell JV, Gomez-Lechon MJ, Grassa C, Martinez LA, Miranda MA, Tarrega P. Photodynamic lipid peroxidation by the photosensitizing nonsteroidal antiinflammatory drugs suprofen and tiaprofenic acid. *Photochem Photobiol*. 1994;59:35-9.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Martinez RM, Pinho-Ribeiro FA, Steffen VS, Caviglione CV, Vignoli JA, Barbosa DS, et al. Naringenin Inhibits UVB Irradiation-Induced Inflammation and Oxidative Stress in the Skin of Hairless Mice. *Journal of natural products*. 2015;78:1647-55.

Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. *Pharmaceutical research*. 2006;23:156-64.

Valacchi G, Rimbach G, Saliou C, Weber SU, Packer L. Effect of benzoyl peroxide on antioxidant status, NF-kappaB activity and interleukin-1alpha gene expression in human keratinocytes. *Toxicology*. 2001;165:225-34.

Relationship: 1851: Oxidation/denaturation of membrane proteins leads to Inflammatory events
(<https://aopwiki.org/relationships/1851>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive oxygen species generated from photoreactive chemicals leading to phototoxic reactions (https://aopwiki.org/aops/282)	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemicals: This KER applies to a wide range of chemicals. The chemicals generate ROS under exposure to light (290-700 nm) (ICH, 2014, Onoue and Tsuda, 2006).

Sex: This KER applies to both males and females.

Life stages: The relevant life stages for this KER are all life stages after born.

Taxonomic: This KER mainly applies to human.

Key Event Relationship Description

Oxidation/denaturation of membrane proteins can lead to inflammatory events via oxidative damage of cellular membranes and increases in expression of pro-inflammatory cytokines.

Necrosis in living tissues can be occurred as an inflammatory events via oxidative damages of membrane proteins.

Evidence Supporting this KER

Biological Plausibility

Oxidative damage of membrane proteins by photoactivated chemicals can cause oxidation and denaturation of proteins, and damaged proteins induces inflammatory events (necrosis) (Dalle Carbonare and Pathak, 1992, Opie, 1962).

Empirical Evidence

Denaturation of proteins induced necrosis and inflammatory in the skin (Opie, 1962).

References

Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. *J Photochem Photobiol B*. 1992;14:105-24.

ICH. ICH Guideline S10 Guidance on Photosafety Evaluation of Pharmaceuticals.: International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2014.

Onoue S, Tsuda Y. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. *Pharmaceutical research*. 2006;23:156-64.

Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. The Journal of experimental medicine. 1962;115:597-608.