



Review article

Chemical photoallergy: photobiochemical mechanisms, classification, and risk assessments



Satomi Onoue^{a,*}, Yoshiki Seto^a, Hideyuki Sato^a, Hayato Nishida^b, Morihiko Hirota^b, Takao Ashikaga^b, Anne Marie Api^c, David Basketter^d, Yoshiki Tokura^e

^a Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

^b Shiseido Global Innovation Center, Shiseido Co. Ltd., 2-2-1 Hayabuchi, Tsuzuki-ku, Yokohama-shi, Kanagawa, 226-8553, Japan

^c Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, USA

^d DABMEB Consultancy Ltd., 2, Normans Road, Sharnbrook, Bedfordshire MK44 1PR, UK

^e Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan

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ABSTRACT

Chemical photosensitivity can be elicited by exposure of the skin to various pharmaceutical substances, foods, cosmetics and other environmental chemicals, followed by exposure to sunlight. There are at least three types of chemical photosensitivity, i.e., photoirritancy (narrowly defined as phototoxicity), photogenotoxicity and photoallergenicity, and their clinical characteristics and mechanisms are quite different. Concerns about chemical photoallergy is increasing, and various studies have been made to clarify the photobiochemical characteristics of photoallergens and the mechanisms involved. Various methodologies, including *in silico* prediction models, photochemical assay systems, and *in vitro* phototoxicity prediction tools, have been developed to predict the photoallergenic potential of chemicals over the past few years. The aim of this manuscript is to review the clinical characteristics, pathogenetic mechanisms and photobiochemical features of photoallergens, with special emphasis on the current status about development of screening systems for predicting photoallergenic potential of chemicals.

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Abbreviations: 3T3 NRU PT, 3T3 neutral red uptake phototoxicity testing; AOP, adverse outcome pathway; DEREK, deductive estimation of risk from existing knowledge; HOMO-LUMO gap, energy gap between the highest occupied molecular orbital and the lowest unoccupied molecular orbital; ICH, International Council for Harmonization of Technical Requirements for Pharmaceuticals for human use; IFRA, International Fragrance Association; LLNA, local lymph node assay; MEC, molar extinction coefficient; mROS assay, micellar ROS assay; NSAIDs, non-steroidal anti-inflammatory drugs; PABA, *p*-aminobenzoic acid; photo-h-CLAT, photo human cell line activation test; PIF, photoirritation factor; QSAR, quantitative structure-activity relationships; ROS, reactive oxygen species; UV, ultraviolet; VIS light, visible light.

* Corresponding author.

E-mail address: onoue@u-shizuoka-ken.ac.jp (S. Onoue).

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1. Introduction

Chemical phototoxicity is an undesirable skin response caused by the combined effect of external agents (such as drugs, cosmetics and food components, even if they are not intrinsically toxic) and exposure to light, especially in the ultraviolet (UV) A and B (320–400 nm and 290–320 nm, respectively) and visible (400–700 nm) wavelengths [1–3]. Based on the types of skin responses and their mechanisms, chemical phototoxicity can be categorized into three main groups: photoirritancy (a narrowly specified type of phototoxicity), photogenotoxicity, and photoallergenicity [4–6]. Among them, photoirritation is most common and can occur in anyone exposed to sufficient amounts of phototoxins and appropriate wavelengths of UV light (Table 1).

Over the past few years, various *in vitro/in vivo* assay systems have been developed for photosafety assessment both in industry and in academia, and guidance on photosafety testing of medicinal products was established by regulatory agencies in the US and EU in the early 2000s [3]. The International Council on Harmonization (ICH) S10 guidelines on photosafety evaluation reached step 5 of the ICH process in 2014, describing detailed photosafety assessment strategies on the basis of photochemical and photobiochemical properties, as well as *in vivo* pharmacokinetic behavior [7]. However, the current ICH S10 guideline “Photosafety evaluation of pharmaceuticals” is focused on the photoirritancy potential of new drug candidates, and risk management of photoallergenicity and photogenotoxicity is currently beyond its scope, because best practice for identification and avoidance of these hazards remains to be established.

Nevertheless, considerable attention has been paid to photoallergenic risk by both industry and regulatory agencies [8–11]. A reliable photoallergy testing system is very important for the early phases of drug discovery and development of cosmetic products, both to minimize the possibility of side effects and to reduce costs by eliminating potentially unsatisfactory products at as early a stage as possible. A better understanding of the pathogenetic mechanisms of chemical photoallergy and the biochemical characteristics of photoallergens would be of great value for development of more reliable photosafety testing tools. In the present review, we focus on the photochemical and photobiochemical features of photoallergens, and on current developments in assay systems for predicting the photoallergenic potential of chemicals, especially newly developed screening strategies.

2. Photoallergic reactions

2.1. Clinical characteristics

Of all phototoxic responses, photoallergy and photoirritation have been of most concern, notable for some topical drugs, cosmetics and nutraceuticals intended for human use. Photoallergy is a delayed immune response to photosensitizer-bound proteins [12], and the toxic event was first reported in the early 1960s in association with the use of halogenated salicylanilides [13]. In contrast, photoirritation, a narrowly specified type of phototoxicity, can be defined as a more immediate and direct inflammatory event in UV-exposed tissues, triggered by photo-oxidation of lipids and proteins in the cellular membrane (Table 1) [8,9]. The dose of both chemical and radiation required for phototoxic responses is usually considered to be more critical to the induction of photoirritation as compared with photoallergy, and the amount of drug required for photoallergic reactions is considerably smaller than that required for photoirritation. Chemical photoirritation often occurs within minutes to hours of sunlight exposure, and acute photoirritation often begins as an exaggerated sunburn reaction. The onset of a photoallergic reaction is usually delayed for 24 h or even several days, and recovery is often slower than from a photoirritant reaction, with the reaction sometimes persisting for some time after the offending product has been discontinued [10]. Photoallergy has also been reported with certain fluoroquinolones, sometimes with cross-reaction to other related-chemicals [10,14]. Histologically, the immediate wheal-and-flare response may show vasodilation and edema, which are difficult to be detected. Delayed reactions present a number of microscopic findings; eczematous eruption with erythema, papules and vesicles, pruritis, weeping, oozing and crusting, and later, scaling and lichenification. In contrast, histologic examination of biopsies from skin in patients with photoirritation reveals necrosis of keratinocytes. Hyperpigmentation does not occur in photoallergic reactions [11].

Several chemicals exhibit both photoirritancy and photoallergenicity, and it can be clinically difficult to distinguish between photoirritation and photoallergy [8–11]. In most cases, such distinctions are usually based on predictive animal and *in vitro* studies, not on a clinical basis. Therefore, further information and careful investigation of patients would be essential before

Table 1
Typical features of photoallergic and phototoxic reactions in skin.

	Photoallergic reactions	Phototoxic reactions
Relative incidence	Low	High
Reaction after first exposure	No	Yes
Onset of reactions	24–72 h	Minutes to hours
Amount of agent required	Small	Large
Dose dependence		
Chemical	Not crucial	Important
Radiation	Not crucial	Important
Action spectrum	Wide, usually UVA	Narrow, long UVA
Clinical appearance	Spongiotic dermatitis	Exaggerated sunburn, edema
Tendency to spread	Yes	No
Pathophysiology	Type IV delayed hypersensitivity	Direct tissue injury
Pigmentary alteration	None	Often
Cross reactivity with other agents	Often	None

attributing the label of photoallergy to an agent known to be capable of photoirritancy.

2.2. Photochemical properties and photoreactions of photoallergens

Absorption of light through the skin varies with wavelength: for example, light in the red region of the spectrum penetrates well into the subcutis layer; whereas at 300 nm or shorter wavelength, only an estimated 10% of incident light passes through the epidermis [15]. When a drug molecule absorbs a photon energy, electrons can be promoted from occupied orbitals (the ground state) to an unoccupied orbital (S_1 , S_2), depending upon the bond type and associated energy level (Fig. 1A). Unpaired singlet state electrons (opposite spin) may be converted to a triplet state (parallel spin) by inversion of the spin *via* intersystem crossing of the absorbed energy. Absorbed energy can be dissipated by internal conversion, fluorescence (from a singlet state), phosphorescence (from a triplet state) or *via* chemical reaction, giving rise to photoproducts and/or intermediates that are potentially reactive with other molecules, including various biomolecules. 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) lies at a comparatively low energy. Energy transfer from an excited triplet photosensitizer to oxygen (type II photochemical reaction) could produce excited singlet oxygen which might, in turn, participate in oxidation of membrane lipids and proteins, or induce DNA damage (Fig. 1B). Electron or hydrogen transfer could lead to the formation of free radical species (type I photochemical reaction) that can react with biomolecules either directly or in the presence of oxygen, forming secondary free radicals such as peroxy radicals or the very reactive hydroxyl radical, a known intermediate in the oxidative damage of DNA and other biomolecules. These direct and/or indirect photochemical reactions of excited photosensitizers may lead to (i) photoirritation through oxidative damage to cellular lipids and proteins, (ii) photogenotoxicity through DNA damage, and (iii) photoallergy through formation of photoantigens [6,16]. These phototoxic reactions could be induced simultaneously by some chemicals, and the photochemical reaction of some compounds with reactive oxygen species (ROS) also resulted in the yield of some toxic degradants [6,17].

2.3. Biochemical mechanisms of chemical photoallergy

Photoallergic responses are considered to be T-cell-mediated hypersensitivity processes requiring pre-sensitization (Fig. 2) [18]. After the distribution or penetration of a photoallergens into the skin, exposure to light can form a reactive species that acts as a hapten, covalently binding to protein to generate a complete antigen in the skin. Once the antigenic hapten-protein complex is formed, epidermal dendritic cells can present it to immunocompetent cells, causing hypersensitivity with lymphocytic infiltration, release of lymphokines, activation of mast cells, and increased cytokine expression. Thus, T cells become activated with transformation into allergen-specific T cells, proliferate, and return to the site of photoallergen deposition, leading to an inflammatory skin response. There are at least two types of photoallergic reactions: delayed hypersensitivity responses and, more rarely, immediate hypersensitivity reactions due to an IgE response to UV [19–21]. An incubation period for the immunologic memory to develop after the first contact with the photosensitizer is required, so there is no reaction on first exposure. On subsequent exposures, the elicitation

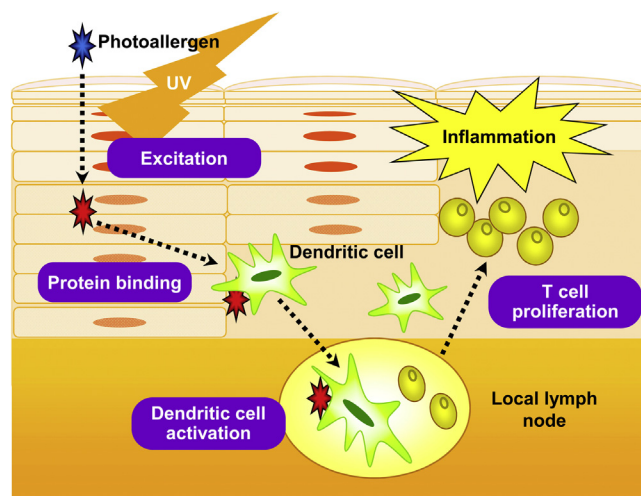


Fig. 2. Photochemical and immunological mechanisms of chemical photoallergy.

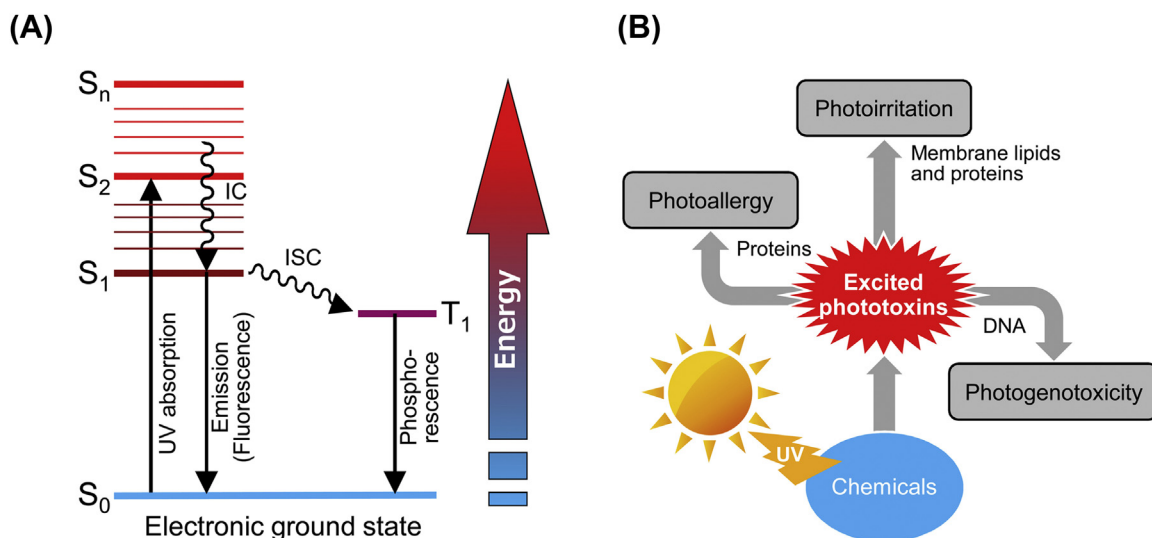


Fig. 1. Possible mechanisms of drug-induced phototoxicity. (A) Jablonski diagram. S: singlet state; T: triplet state; IC: internal conversion; and ISC: intersystem crossing. Lines within singlet states indicate excited vibrational states; excited rotational states are not shown. Reprinted with some modifications from Onoue et al. [3], Copyright (2009), with permission from Bentham Science Publishers. (B) Several phototoxic responses caused by photo-activated drugs.

of response is faster [19]. Thus, the pathogenesis of chemical photoallergy involves an induction (sensitization) phase and an effector (elicitation) phase, and a clear understanding of both processes is needed to design reliable prediction tools and de-risking strategies for the early development stages of drugs, cosmetics and nutraceuticals.

3. Photoallergens

3.1. Pharmaceutical substances

Several classes of chemicals, including antibiotics, antifungals, antihistamines, cardiovascular drugs and NSAIDs, cause photoallergic skin responses (Table 2) [10,22–29]. Most photoirritant reactions result from systemic administration of the agent, whereas photoallergic reactions may be caused by either topical or systemic administration, with the onset of the reaction usually being delayed for 24 h to several days [27].

A number of widely used medications are associated with chemical photoallergy, and many photoallergens simultaneously exhibit photoirritation as well. It would be highly challenging to discriminate photoallergy and photoirritation, and both can be mixed to various extents in clinical setting. The frequency with which individual medications evoke the photoallergic responses appears to be quite variable in the human populations [10]. NSAIDs are one of the most commonly incriminated photoallergens [30], although NSAIDs were originally designed to alleviate inflammatory responses. Particular culprits include diclofenac, ketoprofen, piroxicam and thiaprofenic acid [31], and photochemical and photobiological investigations of NSAIDs have shown that 2-arylpropionic acid derivatives, such as ibuprofen and ketoprofen, are the most photoactive. Photocontact allergy to ketoprofen also induces photo cross-reactions to a number of structurally similar pharmaceutical and cosmetic chemicals [13]. In addition, antibiotic fluoroquinolones are well-known to cause photosensitivity, and the photoallergenicity of quinolone derivatives such as lomefloxacin and enoxacin is mainly derived from their photo-haptenic moiety [10,14]. There appears to be broad photoantigenic cross-reactivity among fluoroquinolones, and patients might develop photoallergy even to a first administration of fluoroquinolones if they had been previously sensitized with other quinolone derivatives. Some antifungals, including griseofulvin and ketoconazole, are also potent phototoxins that can cause severe photoallergic responses [12,25,27].

3.2. Cosmetics

In addition to pharmaceuticals, some cosmetic ingredients exhibited potent photoallergenic potential, as well as photo-irritancy. Cosmetic products are often designed to remain on the skin despite rinsing, and repeated applications of phototoxic cosmetics to the skin would therefore increase the risk. Cosmetic use of 6-methylcoumarin, musk ambrette and hexachlorophene has already declined significantly after use of these materials were discontinued, following numerous reports on their photoallergic potential [23]. Nowadays, cosmetic photoallergenicity is most frequently associated with sunscreens containing *p*-aminobenzoic acid (PABA) derivatives, benzophenones, salicylates, dibenzoylmethane derivatives, and anthranilates [32]. Many reports of photoallergy to PABA derivatives have led to their near removal from current sunscreen manufacturing. Benzophenones are widely used in sunscreens and hair sprays, and some benzophenone derivatives have structural similarities with ketoprofen, resulting in considerable cross-reactivity [13]. A number of fragrance ingredients have been associated with photoallergic responses. For example, musk ambrette and 6-methylcoumarin are typical

synthetic fragrances that caused an epidemic of photoallergic contact dermatitis [23,25,28]. Both musk ambrette and 6-methylcoumarin are no longer used as fragrance ingredients. They have been prohibited from use as fragrance ingredients by the International Fragrance Association (IFRA) 1981 since 1979, respectively. Some antibacterials also show photoallergic responses [23,25,28,29]. Triclosan has been used as antibacterial agent in bar soap and deodorants, and it appears to be a very low-level photosensitizer [29]. Fenticlor is a chlorinated phenol used as an antibacterial and antiseborrheic agents in hair-care products, and it has been recognized as a moderately potent photoallergen [23,25,29].

Despite careful screening before new product launches, it is likely that new photoallergens will continue to emerge. Clinicians should remain alert to this possibility, and manufacturers should conduct follow-up surveys after new product launches to ensure early identification of any issues.

4. Risk assessments

4.1. Predictive tools

Various assessment tools for evaluating phototoxic potentials of chemicals have been developed over the past few years, based on the pathogenetic mechanisms of drug-induced phototoxicity (Table 3). Although multiple photoirritation testing tools have been developed and validated so far, there are still few assay systems for chemical photoallergy. Available tools include *in silico* prediction systems [33–35] for deductive estimation of risk from existing knowledge (DEREK) and from the energy gap between the highest occupied molecular orbital and the lowest unoccupied molecular orbital (HOMO-LUMO gap). DEREK allows toxicity prediction of chemicals based on structures known to be associated with the incidence of toxicity [33], and HOMO-LUMO gap evaluation provides a measure of the photoreactive potential of chemicals [34]. The photochemical properties of tested chemicals are also a key indicator of the phototoxic potential: UV spectral analysis can provide reliable information on the photoexcitability of chemicals [36]. ROS assay and micellar ROS (mROS) assay were also proposed for evaluating the phototoxic risk of chemicals, since photo-activated phototoxins tended to generate ROS, such as singlet oxygen and superoxide [16,37]. Both UV spectral analysis and ROS assay are validated and thereby recommended in the ICH S10 guideline [7]. Although these *in silico* and photochemical prediction tools are not completely specific for chemical photoallergy, the prediction methods, perhaps deployed in a high throughput system, may be appropriate for initial screening of chemicals to predict risk of photoallergenicity.

In addition to these assay systems, several *in vitro* screening systems, such as a pig skin model and a photo human cell line activation test (photo-h-CLAT), have been developed for evaluating the photoallergic potential [38–42]. The pig skin model evaluates the formation of drug-protein photoadducts in pig skin [38], and photo-h-CLAT monitors the overexpression of CD86/54 on the dendritic-like cells associated with antigen presentation to T lymphocytes [39]. Another model is the photo-SH/NH₂ test, which was designed to monitor changes of cell-surface thiols and amines on human monocytic THP-1 cells, since these functional groups are indispensable for covalent binding of activated photoallergens with proteins [42]. As a clinical inspection tool, an *in vivo* photopatch test has been employed for evaluating drug-induced phototoxicity on the basis of both photoirritant and photoallergic reactions in the skin [43]. For more detailed evaluation of photoallergic potential, local lymph node assay (LLNA) was developed, in which the immune reactions in the draining lymph nodes are measured [44]. Photoallergic potential has also been

Table 2
Causative photoallergens.

Classes/Chemicals	Photosafety information		Reference
	Photoallergy	Photoirritation	
Anesthetic			
Dibucaine	+		[24]
Antibiotics/Antimicrobials			
Chlorhexidine	+		[25,26]
Dapsone	+	—	[26,28]
Doxycycline	+	+	[12,24]
Enoxacin	+	+	[12,23]
Fleroxacin	+	+	[12]
Isoniazid	+		[12,22,24]
Lomefloxacin	+	+	[12,24]
Moxifloxacin	+	+	[24]
Nalidixic acid	+	+	[24]
Ofloxacin	+	+	[24]
Omadine	+		[22]
Sparfloxacin	+	+	[12]
Sulfanilamide	+		[22,23]
Tetracycline	+	+	[24]
Anticancer agents			
Dacarbazine	+	+	[12,24]
Flutamide	+		[12,24]
Vemurafenib	+	+	[12,24]
Anticonvulsants			
Carbamazepine	+		[12,24]
Antidepressant			
Imipramine	+		[24]
Antidiabetics			
Glibenclamide	+		[22]
Glipizide	+	+	[27,28]
Antifungals			
Griseofulvin	+	+	[12,25,27]
Itraconazole	+	+	[27,28]
Ketoconazole	+	+	[24,27]
Voriconazole	+	+	[24]
Antihistamines			
Diphenhydramine	+		[10,24]
Mequitazine	+		[12,24]
Promethazine	+	+	[23,25,27]
Antimalarials			
Chloroquine	+	+	[26]
Quinine	+	+	[23,25,26]
Antirheumatic drugs			
Sulfasalazine (Salazosulfapyridine)	+		[22]
Antivirals			
Acyclovir	+		[24,26]
Cardiovascular drugs			
Amiodarone	+	+	[24]
Quinidine	+	+	[27–29]
Diuretics			
Furosemide	+	+	[12,24]
Hydrochlorothiazide	+	+	[12,22]
Meticrane	+		[12]
Hypotensive agents			
Tilisolol	+		[12,24]
Muscle relaxants			
Afloqualone	+		[12]
Neuroleptics			
Chlorpromazine	+	+	[23,25,27]
Prochlorperazine	+		[24]
NSAIDs			
Benzydamine	+		[24]
Celecoxib	+	—	[24,26,27]
Diclofenac	+	+	[23,25]
Etofenamate	+		[24]
Flufenamic acid	+		[24]
Ibuprofen	+		[24]
Ketoprofen	+	+	[23,25,27]
Mesalazine	+		[24]
Piketoprofen	+		[24]
Piroxicam	+	+	[22,23,29]
Suprofen	+		[24]
Tiaprofenic acid	+		[12,24]
Cosmetics/Nutraceuticals			
4-Methyl-7-ethoxycoumarin	+		[23]
6-Methylcoumarin	+	+	[23,25,28]

Table 2 (Continued)

Classes/Chemicals	Photosafety information		Reference
	Photoallergy	Photoirritation	
7-Methoxycoumarin	+		[23]
8-Methoxypsoralen	+	+	[23]
Avobenzene	+		[29]
Benzophenone	+	+	[23,28,29]
Bithionol	+	+	[23,29]
Dichlorophene	+		[23]
Fenticlor	+	+	[23,25,29]
Hexachlorophene	+	–	[23,25,29]
Musk ambrette	+	–	[23,25,28]
Musk xylene	+	–	[23]
PABA derivatives (e.g. Octyl dimethyl PABA)	+	–	[22]
p-Phenylenediamine	+		[23]
Pyridoxine	+	+	[12,22]
Tetrachlorosalicylanilide	+	+	[23,29]
Tribromsalan	+	+	[22,23,29]
Triclocarban	+	–	[23]
Triclosan	+		[29]

Table 3

Assay approach for testing photoallergenic potential.

Assessments	§ Assay principle	References
<i>In silico</i> prediction		
DEREK	Structure-based photosafety prediction	Barratt et al. [33]
HOMO-LUMO gap	Energy differences between levels of HOMO and LUMO	Betowski et al. [34]
QSAR model	Structure-based photosafety prediction	Ringeissen et al. [35]
Photochemical properties		
UV-vis spectral analysis	UV-vis absorption of chemicals	Henry et al. [36]
ROS assay	Generation of ROS (singlet oxygen and superoxide) from irradiated chemicals	Onoue et al. [16]
mROS assay	Generation of ROS (singlet oxygen and superoxide) from irradiated chemicals	Seto et al. [37]
<i>In vitro</i> photoallergy		
Pig skin model	Formation of drug-protein photoadduct in pig skin	Sarabia et al. [38]
Photo-h-CLAT	Human monocytes activation by irradiated chemicals	Hoya et al. [39]
NCTC2455 assay	Interleukin (IL)-18 production from keratinocytes by irradiated chemicals	Galbiati et al. [40]
Dendritic cell-based assay	Dendritic cell activation by irradiated chemicals	Karschuk et al. [41]
Photo-SH/NH ₂ test	Changes of cell-surface thiols and amines by irradiated chemicals	Oeda et al. [42]
<i>In vivo</i> photoallergy		
Photopatch test	Photoallergic skin responses by topical oxidative stress	Epstein [43]
Mouse ear-swelling model	Measurement of ear swelling	Gerberick and Ryan [45]
Local lymph node assay	Proliferation of lymphocytes in the lymph nodes	Vohr et al. [44]
Photomaximization test	Photoallergic skin reactions by irradiated chemicals	Kaidbey and Kligman [46]

assessed by means of a mouse ear-swelling model [45] and a photomaximization test [46], in which photoallergic response is determined upon a subjective evaluation of erythema and edema at the irradiated sites in human volunteers. However, outcomes from these phototestings might be affected by multiple factors, including the species, light source and dose, purity of tested chemicals, and the nature of the exposure to tested chemicals (dosage form, route of administration, and dosing regimen) [47]. Therefore, careful consideration of the experimental conditions and obtained outcomes should be made in order not to provide incorrect predictions.

4.2. Predictive capacity of validated assays for photoallergenicity

In 2014, ICH S10 guidelines on photosafety evaluation reached step 5 of the ICH process [7], in which three *in vitro* photosafety test methods are recommended: (i) UV spectral analysis [36], (ii) ROS assay [16], and (iii) 3T3 neutral red uptake phototoxicity test (3T3 NRU PT) [48]. Outcomes from UV spectral analysis and ROS assay can be potential indicators of photoreactivity, and 3T3 NRU PT was established to assess the cytotoxicity of photo-excited chemicals as

an *in vitro* alternative to *in vivo* phototoxicity tests. These tests were not originally designed for specific prediction of chemical photoallergenicity; however, many photoallergens can be captured by these screening systems as phototoxins, which may cause both photoirritation and photoallergy. To clarify the predictive performance of these validated assays in terms of photoallergic potential, comparative photosafety testing was previously carried out for 23 photoallergens and 7 non-phototoxic/non-photoallergenic chemicals (Table 4) [22]. UV/vis spectral analysis correctly identified ca. 96% of tested photoallergens, with a few false-positive predictions for non-phototoxic chemicals. In the ROS assay, generation of superoxide by some irradiated photoallergens was negligible; however, if both singlet oxygen and superoxide were measured, all photoallergens were correctly predicted with the positive and negative predictivities of 95.8% and 100%, respectively. Although most of the photoirritant chemicals were correctly identified by the 3T3 NRU PT, it provided false predictions for almost half of the photoallergens group. Thus, these assay systems were found to be applicable at least to some extent for prediction of photoallergenicity, and their predictive capacity was ranked as follows: [Sensitivity]: ROS assay (100%)>UV/vis spectral analysis (95.7%)>

Table 4

Photosafety characterization with use of validated assays (Excerpt).

Photoallergens	Maximal MEC (M ⁻¹ cm ⁻¹) ^{a)}	ROS/mROS ^{b)}		3T3 NRU PT (PIF) ^{c)}	Photosafety information	
		Singlet oxygen ($\Delta A_{440\text{ nm}} \cdot 10^3$)	Superoxide ($\Delta A_{560\text{ nm}} \cdot 10^3$)		Photoallergenic	Photoirritant
6-Methylcoumarin	7,502 [290 nm]	196±11	112±8	21.3	+	+
Benzophenone	1,305 [290 nm]	227±21	< 1	10.0	+	+
Bithionol	13,253 [307 nm]	273±22	< 1	3.6	+	+
Hexachlorophene	8,381 [298 nm]	437±5	< 1	1.0	+	–
Indomethacin	8,738 [290 nm]	29±13	168±18	1.5	+	+
Musk ambrette	2,755 [290 nm]	[224±2]	[< 1]	104.2	+	–
Musk xylene	448 [290 nm]	[104±3]	[38±7]	29.1	+	–
Octyl dimethyl PABA	28,879 [310 nm]	[76±3]	[158±9]	2.7	+	–
Piroxicam	22,389 [327 nm]	181±29	204±17	1.0	+	–
Pyridoxine HCl	7,539 [291 nm]	421±2	98±2	3.4	+	+
Sulfanilamide	3,677 [290 nm]	265±19	41±4	1.0	+	–
Sulfasalazine	33,116 [366 nm]	51±10	19±5	1.0	+	–
Triclocarban	3,342 [290 nm]	[63±2]	[< 1]	1.0	+	–

Black cells: over threshold in MEC and ROS/mROS or phototoxicity predicted in 3T3 NRU PT; and gray cells: probable phototoxicity in 3T3 NRU PT. ^{a)} Plain numbers: MEC values at λ_{max} observed between 290 and 700 nm; and italic numbers: MEC values at 290 nm (shoulders). ^{b)} Plain numbers: ROS data at 200 μM ; and plain numbers in brackets: mROS assay at 200 μM . Data represent mean $\pm$ SD of three repeated experiments. ^{c)} PIF: photoirritation factor. Reprinted with some modifications from Onoue et al. [22], Copyright (2016), with permission from Elsevier.

3T3 NRU PT (52.2%), and [Specificity]: 3T3 NRU PT (100%)>ROS assay (85.7%)>UV/vis spectral analysis (57.1%). In short, the 3T3 NRU PT seems less reliable for photoallergenicity prediction, whereas the *in vitro* photochemical assays could predict photoallergenicity with some false positives. These observations are not surprising for several reasons: (i) the photoreactivity of chemicals is a key determinant for initiation of all phototoxic events, (ii) 3T3 NRU PT was originally designed to detect photoirritation, not photoallergy [3], and (iii) 3T3 NRU PT may give false negative predictions for chemicals predominantly absorbing in the UVB range [49]. Overall, it can be concluded that photochemical assays are available for photoallergy testing, although there were still a substantial risk of false positive predictions.

5. Conclusion and prospects

The mechanisms of chemical photoirritation are currently well understood on the basis of abundant data from basic research and clinical studies, and various *in vitro* assay systems have been developed and validated to identify compounds with a high photoirritation potential. Several guidelines on evaluation of chemical photoirritation have already been produced, and there appears to be a decreasing incidence of phototoxic adverse effects among newly developed chemicals. On the other hand, the pathogenesis of chemical photoallergy is not yet fully understood, and currently available screening systems for photoallergy still have limitations. Thus, research needs to be focused on identification of photoallergens and the precise mechanisms of photoallergenicity, with the aim of establishing reliable assay systems for chemical photoallergens. Further mechanistic investigations might provide a theoretical basis for modification of the current assays to improve their applicability and robustness. It is also important to accelerate the development of new *in vitro* photosensitization assays based on the adverse outcome pathway (AOP) of photosensitization. An in-depth understanding of assay limitations through careful validation studies would be of great

value for improving predictive accuracy and avoiding misleading conclusions. Cross-photosensitivity among similar chemical series is still a substantial problem for both clinicians and manufactures. There are several apparent relationships between the chemical structures of some photoallergens and their photosensitivity, and further accumulation of structure-based photosafety information might lead to strategic establishment of reliable DEREK and QSAR models, providing strategic structure-based alerts for photoallergenicity and potential cross-reactivity among co-administered chemicals. The problem of chemical photoallergy is likely to be seen as increasingly important by both regulatory agencies and industry, and the development of reliable screening strategies for photoallergenicity prediction will be central to the efficient development of new chemicals with a high degree of confidence regarding their photosafety.

Conflict of interest

None of the authors have any conflicts of interest associated with this study.

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Satomi Onoue (Ph.D.) is professor in the Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, Japan. He received his BS (1996) and MS (1998) from Okayama University (Japan), and his PhD (2003) from the University of Shizuoka (Japan). He was formerly in charge of pharmaceutical research and development in Pfizer Global Research and Development with international project team responsibility, and he moved to his current position in 2007. His recent studies have focused on (i) de-risking drug delivery systems; (ii) dry powder inhalation systems; and (3) developing tools for predicting phototoxic potential, that was successfully adopted by ICH (ICH S10 guideline “Photosafety evaluation of pharmaceuticals”).