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令和４年度 分担研究報告書

光毒性の AOP 及び IATA の開発

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研究要旨

光線過敏症とは特定の化学物質摂取後、太陽光への曝露によって惹起される皮膚および眼における異常反応である。光線過敏症は医薬品のみならず、食品および化粧品等が原因となる場合も報告されており、新規化合物ならびに製品開発における光毒性リスクの回避は重要な課題となっている。光毒性カスケードの上流においては、化合物の光エネルギー吸収に伴う種々の光化学的イベントが認められ、特に光によって励起された化合物の光化学反応性は光毒性リスクに直接的に関係していると考えられる。本研究では光化学的イベントに焦点を置いた AOP を新規に作成するとともに、AOP を基盤とした IATA の開発を行い、動物実験に依存しない光安全性保障システム構築を指向した。

A. 研究目的

地球上に降り注ぐ太陽光は生態系に多くの恵みを与え、太陽光のうち UVC 領域は DNA 損傷などの有害事象を惹起するものの、高エネルギーな短波長光を除けば本来は人体に対してほとんど無害である。しかし、ヒトに投薬された医薬品が体内で光と相互作用を起こすことによって、主に皮膚や眼において炎症、角質化、色素沈着などの有害反応を誘発することがあり、これを広義の光毒性と定義する。対象となるのが薬剤であった際にはこの有害事象を薬剤性光線過敏症と呼称するが、医薬品以外でもこれまでに多くの食品、化粧品等においても同様の光毒性反応が認められている。近年のオゾン層破壊に伴う紫外線量の著しい増加の背景もあって光毒性リスクへの注目が高まっており、光毒性発症機序解明ならびに評価系開発が精力的に進められている。ICH S10 ガイドラインでは、化合物の i) 光反応性および ii) 露光部位（皮膚や眼）への分布が光毒性発現に重要な因子として明記されている。研究分担者らは先に光化学的評価方法として reactive oxygen species (ROS) assay を開発し、本データと皮膚内動態情報の組み合わせることで信頼性ある光安全性評価が可能となることを明らかにした。さらに ROS assay による光化学的特性および *in vitro* 皮膚内動態のデータを統合的に解析することで経皮適用化合物の光毒性リスクを効果的に予測できることを検証し、その予測データを用いることで動物実験代替法の開発を指向した検討を実施してきた。これらの検証結果を基に光毒性に関する AOP ならび光安全性評価に関する IATA 案構築を試みており、当該年度は AOP の最終化ならびに IATA 案提出ならびに当該領域専門家による review 結果に基づいて修正案作成に従事した。

B. 研究方法

B-1. 光毒性の AOP

研究分担者の尾上は、開発中の光毒性

AOP を専門家の意見に基づいて改変し、AOP wiki を更新した。

B-2. 光毒性 IATA

尾上は光毒性 IATA 案を作成するとともに、OECD expert group からのコメントに従って光毒性 IATA 案を修正した。

C. 研究結果

C-1. 光毒性の AOP

OECD の専門家会議で意見を求め、光毒性反応のうち光刺激性に限定した AOP 作成を推進した (Fig. 1)。体内に取り込まれた光毒性物質はまず皮膚組織に到達し、薬剤の分子内 chromophore、あるいは代謝によって獲得された chromophore が皮膚深部まで到達した光によって照射されると、基底状態の S_0 から励起一重項状態 S_1 に励起される。励起一重項状態の寿命は極めて短く、すなわち蛍光を発して直ちに基底状態 S_0 に戻るか、項間交差により励起三重項状態 T_1 に遷移する。励起三重項状態にある化合物はりん光を発して基底状態 S_0 に戻る。基底状態ではまったく化学反応をしない条件でも、高い光エネルギーを獲得した励起分子は、そのエネルギーを駆動力として結合の解裂や生成または組み換えなどの化学反応を起こすことができる。そのような過程を光化学過程といい、ラジカル反応である Type I 反応と、一重項酸素反応である Type II 反応とに分けられる。酸素分子は励起エネルギーのアクセプターとして機能し、それに伴い産生された singlet oxygen や superoxide 等の活性酸素種 (Reactive oxygen species; ROS) による生体内物質の酸化反応が薬剤性光線過敏症の発症原因の一つとして考えられている。これらの光化学反応の標的が細胞膜上の各種生体成分である場合には光刺激性を誘発し、また DNA の酸化あるいは塩基修飾によって光遺伝毒性や光がん原性が発現する。励起された薬物がハプテンとなりタンパク質と光付加物を形成した際には、免疫原性を示すことになり、最終的に光アレルギー反応を惹起するものと考えられる。いずれにせよ、薬剤性光線過敏症の機序を考えるとき、最も重要なトリガーとなるのは太陽光の吸収、そしてそれに伴う化合物の励起であろう。しかし、励起された全ての化合物が一様に光毒性を惹起するわけではなく、実質的な光化学的反応を引き起こす化合物が光毒性を誘発するものと考える。この観点から「太陽光の吸収しやすさ」の指標である UV/VIS 吸収特性よりも、むしろ励起エネ

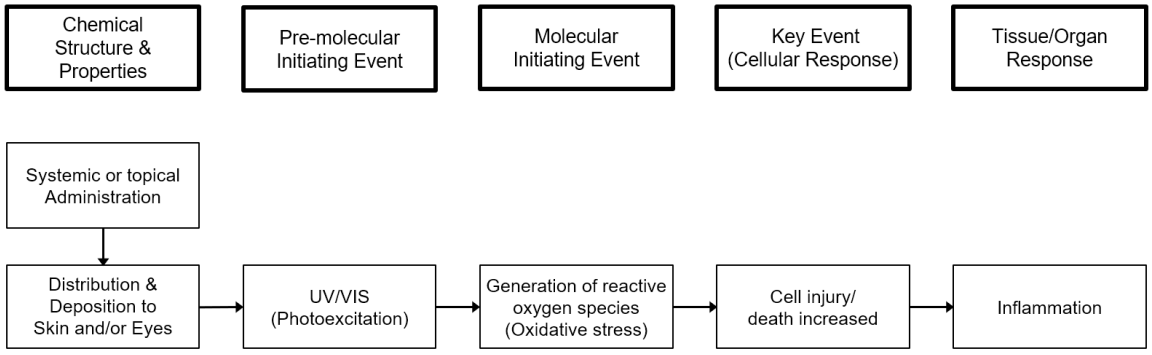

Figure 1. Flow diagram of the adverse outcome pathway and the intermediate steps associated with phototoxic responses.

ルギーによる光化学的反応性を直接評価するアプローチがより実質的な光毒性予測に寄与できる可能性がある。すなわち、光吸収に伴う化学物質の光反応が光毒性のトリガーであるとのコンセプトに基づき、UV/VIS 吸収とそれに伴った励起を pre-MIE として定義した。またそれに伴う MIE は励起化合物からの ROS 産生とし、次いで cell injury を KE、最終的な Tissue response を inflammation とした。

C-2. 光毒性 IATA

作成した AOP を基盤として、その枠組みのなかで information sources を網羅的にマッピングした。光毒性に関与する elements としては、(i) Exposure consideration、(ii) Chemical descriptors、 (iii) Skin penetration、(iv) Photoexcitation、 (v) oxidative stress、 (vi) cell injury を定義し、それらに関わる information sources をリスト化した (Table 1)。具体的な information sources としては既にガイドライン化されているものはもちろんのこと、validation study が実施されていないアッセイ系でも光安全性保障におけるその有用性を考慮して記述した。

これまでに臨床における薬剤性光線過敏症診断としては Photopatch test や皮膚の biopsy 等が従来実施され、特に前者は *in vivo* 光感作試験として幅広く利用されている。一方、創薬においては、簡便に光安全性を評価する方法として *in vitro* 3T3 NRU phototoxicity test が代替試験法として利用されている。しかしながら創薬初期過程ではスクリーニングの更なる高効率化や異なる機序の光線過敏症リスク評価が求められ、これまでにも数多くの評価系が開発・応用されている。これらは、*in silico* スクリーニングツールや、光化学的特性を中心とした分子物性評価、あるいは各種毒性反応に特異的な光生物化学的アッセイ方法などが含まれている。これらのアッセイ手法は創薬のステージによってその利用方法や位置づけが異なっており、例えば DEREK や HOMO-LUMO Gap 等の *in silico* システムでは創薬支援として化合物が実際に合成される前段階で新規化合物の光安全性予測を行うことを目的で利用されている。一方、光化学的特性評価ツールは実際に合成された新規化合物の分子物性を指標とした光感受性分析を主体とし、必ずしも光安全性を直接的に評価可能なわけではないが、光毒性反応の誘発に寄与し得る光化学的反応の有無について高いスループットで示唆することが出来る。また、光刺激性評価のためのツールはこれまでにも数多く開発されており、枯草菌、白血球や赤血球を用いたアッセイ系やヒト三次元培養皮膚モデルを利用した評価系が *in vitro* 光毒性試験法として有用である。 これらの *in vitro* 試験、*in vivo* 試験のみならず、*in silico* や QSAR モデルも information sources に含めることとし、多様な光安全性評価を可能とした。

先に我々は、皮膚内動態情報と光反応性情報を統合することによって光安全性評価の予測精度を向上させることを明らかにしているが、皮膚内動態については被験物質の物理化学的特性からも部分的に予測可能であり、ROS assay データによる光反応性情報との組み合わせによって動物実験に依存しない光安全性保障システムの構築が可能になるものと考える。この観点から薬物動態に関係する各種因子についても網羅的に記述した。また、OECD 専門家会議メンバーによるレビューの結果、各 information source について validation status と weight of importance を加えるよう要望があり、これに基づいて当該情報を追記した。

Table 1. Elements and examples of information sources that can be used within defined approaches and IATAs for phototoxicity

Elements	Information sources addressing each element	Validation status/weight of importance
Exposure consideration	● Applied dose	– / mid-high
	● Frequency of dosing	– / mid
	● Formulation effects	– / mid
	● Route of exposure	– / mid-high
	● Accumulation of compounds in the skin/eyes	– / high
	● *In vitro* to *in vivo* extrapolation	– / mid-high
Chemical descriptors	**Chemical structure**	
	● Structure alert	– / mid-high
	● QSAR model	– / mid-high
	Physicochemical properties	
	● Molecular weight	– / mid
	● pK_a	– / low
	● Partition coefficient (log *P*, log *D*)	– / mid-high
	● Water solubility	– / low
	● *in vitro* membrane permeability	Validated / high
Skin penetration	**Non-testing methods**	
	● Characterization of skin absorption with use of physiologically-based pharmacokinetic models	Validated / high
	Testing methods	Validated / high
	● OECD TG 427 (Skin absorption: *in vivo* methods)	
	● OECD TG 428 (Skin absorption: *in vitro* methods)	Validated / high
AOP Pre-MIE:	● UV/VIS absorption	Validated / high
Photoexcitation	● OECD TG 495 (ROS assay)	Validated / high
	● Photostability testing	– / low-mid
	● Homo-Lumo gap calculation	– / low
AOP MIE:	● Photohemolysis model	– / low-mid
Oxidative stress	● Oxygen consumption in *Bacillus subtilis*	– / low
	● Yeast growth inhibition assay	– / low-mid
	● DNA photocleavage assay	– / low/mid
AOP key event:	● OECD TG432 (3T3 NRU phototoxicity testing)	Validated / high
Cell injury/death increased	● OECD TG498 (*in vitro* reconstructed human epidermis phototoxicity test)	Validated / high

また、OECD 専門家会議における有識者の助言に従い、information source に対して詳細な記述を加えることとし、具体的には (1) Regulatory use、(2) Validation & regulatory acceptance status、(3) Potential role in the IATA、(4) Description、 (5) Scientific basis including MoA、(6) Protocol available、(7) Strengths and weakness 、 (8) Applicability domain and limitations、 (9) Predictive capacity、 (10) Reliability を各種文献情報やガイドラインを交えつつ追記した (Table 2)。全ての information sources に対してこれらの情報を入手するのは容易ではなく、それ故、情報が比較的入手しやすく、なおかつ重要度の高いガイドライン化された光安全性評価系に焦点をあてて記載を試みた。

Table 2. Description of the key elements of the IATA for phototoxicity

(a) UV/VIS absorption

Regulatory use	Identification on photoexcitability of test chemicals by spectroscopic determination of UV/VIS-absorbing properties
Validation & regulatory acceptance status	Validated and adopted as OECD TG 101; presented under guidance document ICH S10.
Potential role in the IATA	Absorption of sunlight by phototoxins, followed by photochemical reaction, is considered to be a key trigger for phototoxicity, because photo-excited chemicals may react with biomolecules, leading to phototoxic events. In this context, the UV-absorbing property of chemicals can be a potential indicator for phototoxic risk, and Henry, et al. demonstrated that chemicals with a molar extinction coefficient (MEC) of less than 1,000 $M^{-1}cm^{-1}$ showed low phototoxic risk.
Description	Each chemical is dissolved in distilled water or appropriate organic solvent at several concentrations (e.g., 0.001, 0.01 and 0.1 μM), and the final concentration can be reduced if the tested chemical is found to be an intense UV/VIS absorber. UV/VIS absorption spectra (290–700 nm) are recorded with a spectrophotometer interfaced to a PC for data processing. MEC values can be calculated from maximum absorbance at several concentrations.
Scientific basis including MoA	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. When a chemical absorbs 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. 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, potentially leading to various

	phototoxic symptoms.
Protocol available	Experimental protocol was established by Henry, *et al.* and Bauer, *et al.*
Strengths and weakness	Strengths - This *in chemico* test method offers rapid, reproducible and high-throughput (i.e., using 96-well method approaches) results. - Test chemicals that do not show significant absorbance (e.g. MEC >1000 M-1cm-1) may not need further photosafety evaluation Weakness - Some chemicals can be UV/visible light absorbers but do not pose phototoxicity hazard or risk, 'positive' prediction from this method needs to be further evaluated with subsequent testing methods. - Standardized conditions for determination of the MECs are critical. Selection of an adequate solvent is driven by both analytical requirements (e.g., dissolving power, UV-visible light transparency) and physiological relevance (e.g., pH 7.4-buffered aqueous conditions).
Applicability domain and limitations	Applicability - The test method is applicable to substances. Limitations - It may not be possible to evaluate poorly-water soluble chemicals in this *in chemico* test method. - The limitations of the chosen method need to be considered (e.g., linear range of the experimental set up). Potential artifacts (e.g., due to concentrations that are too high or precipitating) has to be carefully assessed. - For calculation of MEC, defined molecular weight of test chemical is needed, so that it is challenging to apply this test method to complex materials/chemical without defined molecular weight.
Predictive capacity	Henry, et al. demonstrated that all 35 phototoxins tested had absorbance intensities significantly above an MEC threshold of 1,000 L mol^{-1} cm^{-1}. Bauer, et al. verified the predictive performance of MEC threshold (1,000 L mol^{-1} cm^{-1}) with 76 chemicals.
Reliability	When measuring MEC values of 76 chemicals in 6 laboratories, all chemicals were found to have agreement of classification between laboratories.

(b) ROS assay

Regulatory use	Identification on photoreactivity of test chemicals by determination of ROS generation from irradiated chemicals
Validation&regulatory acceptance status	Validated and adopted as OECD TG495; presented under Guidance Document ICH S10.

Potential role in the IATA	The primary event in any photosensitization process is the absorption of photons of the appropriate wavelength, which allows chromophore to reach an excited state. The excitation energy is often transferred to oxygen molecules, followed by generation of ROS: superoxide through type I reaction and singlet oxygen through type II reaction by photo-excited molecules. These appear to be the principal intermediate species in the phototoxic response. The ROS assay can monitor generation of ROS, such as singlet oxygen and superoxide, from photoirradiated chemicals; therefore, the ROS data can be used to evaluate the photoreactivity of chemicals.
Description	In the ROS assay, generation of singlet oxygen was detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows; Singlet oxygen + Imidazole → [Peroxide intermediate] → Oxidized imidazole [Peroxide intermediate] + RNO → RNO + Products The generation of superoxide could be determined by the reduction of nitroblue tetrazolium (NBT) as indicated below; NBT can be reduced by superoxide anion via a one-electron transfer reaction, yielding partially reduced (2 e^-) monoformazan (NBT^+) as a stable intermediate. Thus, superoxide can reduce NBT to NBT^+, whose formation can be monitored spectrophotometrically at 560 nm. Superoxide + NBT → O_2 + NBT^+
Scientific basis including MoA	In any type of phototoxic event, penetration and absorption of light in the skin, eyes, or other UV-exposed tissues can be a critical factor for triggering phototoxic cascades, and the absorption of photon energy by the phototoxin results in excitation of the molecule itself. Since molecular oxygen can act as the predominant acceptor of excitation energy, energy can be transferred from photo-excited chemicals to oxygen through type II photochemical reaction, resulting in the generation of singlet oxygen. Transfer of an electron or hydrogen could also lead to the formation of free radical species such as superoxide, peroxyl radicals or reactive hydroxyl radical through a type I photochemical reaction. Thus, photo-excitation of chemicals tends to produce ROS, which may be one of major causative agents of phototoxic events.
Protocol available	OECD TG495
Strengths and weakness	<u>Strengths</u> - This *in chemico* test method offer rapid and reproducible photosafety

	prediction. - For this test method, UVB light source can be used, that is usually excluded in the cell-based photosafety testing. Weakness - To avoid spectral interference of discoloring chemicals in ROS determination, an experimental control has to be employed upon exposure of tested chemical alone to simulated sunlight, to subtract control readings from sample readings. - In theory, the ROS assay can provide highly sensitive predictions (i.e., false positives), since it may capture all photochemically active substances. Some photolabile substances would be judged as positive in the ROS assay if they are potent ROS generators in their photodegradation pathways.
Applicability domain and limitations	Applicability - The test method is applicable to substances. Limitations - The poorly-water soluble chemicals might be untestable by this *in chemico* test method. In such a case, the mROS assay is available partly. In the mROS assay, Tween20 is added to solvent system, and the formed micelle can enhance the solubility of most test chemicals. - The chemicals with potent chromophores (e.g., rose bengal) might be untestable because of spectral interference.
Predictive capacity	The validation study was previously undertaken to verify the applicability of different solar simulators and assay performance [43, 44]. In 7 participating laboratories, 2 standards and 42 coded chemicals, including 23 phototoxins and 19 non-phototoxic drugs/chemicals, were assessed by the ROS assay using two different solar simulators (ss-1 and -2). In both solar simulators, the intra- and inter-day precisions (coefficient of variation; CV) for quinine were found to be below 10%. The inter-laboratory CV for quinine averaged 15.4% (ss-1) and 13.2% (ss-2) for singlet oxygen and 17.0% (ss-1) and 7.1% (ss-2) for superoxide, suggesting high inter-laboratory reproducibility even though different solar simulators were employed for the ROS assay. In the ROS assay on 42 coded chemicals, some chemicals (ca. 19–29%) were unevaluable because of limited solubility and spectral interference. Although several false positives appeared with positive predictivity of ca. 76–92% (ss-1) and ca. 75–84% (ss-2), there were no false negative predictions in both solar simulators.
Reliability	Multi-center validation study on the ROS assay demonstrated satisfactory transferability, accuracy, precision, and predictivity, as well as the availability of other solar simulators.

(c) 3T3 NRU phototoxicity testing

Regulatory use	Identification of phototoxicity potential of test chemicals using Balb/c 3T3 cultures
Validation&regulatory acceptance status	Validated and adopted as OECD TG 432; presented in ICH S10 guidance document
Potential role in the IATA	The 3T3 NRU PT assesses the cytotoxic effect of a test substance after exposure to a non-cytotoxic dose of UVA light compared with that in the absence of exposure, and the cytotoxicity is expressed as a concentration-dependent reduction of the uptake of the vital dye. Chemicals identified as positive in this test may be phototoxic *in vivo*, following topical application or systemic application and distribution to the UV-exposed tissues.
Description	The cells are exposed to a test chemical in the presence (+Irr) (dose of 5 J/cm^2 of UVA) or absence (-Irr) of UVA light, and viability is assessed 24 hours later by spectrophotometric measurement of neutral red dye uptake by the compound treated cells compared to vehicle treated controls. Chlorpromazine is used as positive control, while Earle's Balanced Salt Solution or other buffered solution may be used as negative controls. The concentration of test article causing a 50% reduction in neutral red dye uptake (IC_{50}) reflects cytotoxic potential. The phototoxic potential is also expressed through the use of two different indices: Photoirritancy Factor (PIF) and Mean Photo Effect (MPE). The PIF is determined by comparing the IC_{50} +Irr to the IC_{50} -Irr and by definition is only useful when IC_{50} values can be determined both with and without UVA exposure. The MPE is determined by comparing the two concentration response curves (-Irr and +Irr) over the range of active test article doses. With respect to phototoxicity prediction on the basis of the results from 3T3 NRU PT, three cases may be considered: 1) a test article with a PIF ＜2 or an MPE ＜0.1 predicts "no phototoxicity"; 2) a test article with a PIF ＞2 and ＜5 or an MPE ＞0.1 and ＜0.15 predicts "equivocal phototoxicity"; and 3) a test article with a PIF ＞5 or an MPE ＞0.15 predicts "phototoxicity".
Scientific basis including MoA	The 3T3 NRU PT is conducted using Balb/c 3T3 mouse fibroblasts to assess the phototoxicity potential of a test article. The assay quantitatively determines the cytotoxic potential of a test article by comparing the reduction in neutral red dye uptake in Balb/c 3T3 cultures exposed to the serial dilutions of a test article, to the neutral red dye uptake in control (the test article vehicle). Phototoxins can induce cell damage through formation of ROS and other mechanisms that lead to increased permeability of the lysosomal membrane, reduction in the pH gradient, and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of neutral red dye. It is thus possible to

	distinguish between viable and damaged or dead cells.
Protocol available	OECD TG432
Strengths and weakness	Strengths - The assay quantitatively determines the cytotoxic potential of a test chemical. - High throughput assay; can screen large numbers of test chemicals for phototoxicity potential - High negative predictivity (further photosafety testing is generally not warranted for test chemicals which are not predicted to have phototoxicity potential in this test method) Weakness - Highly sensitive assay. Detection level is far more sensitive than the magnitude of biological effect. - In the 3T3 NRU PT, a UVA light source with filter to attenuate UVB is used since 3T3 cells are not tolerant to higher doses of UVB light, so the 3T3 NRU PT may provide false-negative prediction for chemicals predominantly or solely absorbing in the UVB range.
Applicability domain and limitations	Applicability - The test method is applicable to substances and mixtures. Limitations - The poorly-water soluble chemicals might be untestable. - In the 3T3 NRU PT, UVB radiation is generally attenuated since cells were killed by UVB radiation; therefore, chemicals excited by only UVB exposure produce false-negative results in the assay.
Predictive capacity	In the validation study, 20 test chemicals were selected and assessed by 3T3 NRU PT. An almost perfect correlation of in vitro versus in vivo results was obtained (between 95% and 100%), when either PIF or MPE were used to predict the phototoxic potential.
Reliability	The 3T3 NRU PT was developed and validated under the auspices of ECVAM from 1992–1997, to establish a valid *in vitro* alternative to the various *in vivo* tests in use. A second validation study was also carried out in 1997 to evaluate the method specifically in terms of selected UV filter chemicals. ESAC subsequently endorsed the validity of the test with respect to these chemicals.

(d) *in vitro* reconstructed human epidermis phototoxicity test

Regulatory use	Identification of phototoxic potential of test chemicals using reconstructed human epidermis phototoxicity test (RhE PT)
Validation & regulatory acceptance status	Validated and adopted as OECD TG498; also presented in guidance document ICH S10
Potential role in the IATA	The *in vitro* RhE PT can be used to identify the phototoxic potential of a test chemical after topical application in RhE tissues in the presence and absence of simulated sunlight. Phototoxicity potential is evaluated by the relative reduction in viability of cells exposed to the test chemical in the presence as compared to the absence of simulated sunlight. Chemicals identified as positive in this test may be phototoxic *in vivo* following topical application to the skin, eyes, and other external light-exposed epithelia. Complementary to cell monolayer phototoxicity tests, this 3-D model allows the topical application of a large panel of chemicals with different physicochemical properties as water insoluble or extreme pH values chemicals, finished products or complex formulations.
Description	Several concentrations of test chemical prepared in a solvent are applied topically to RhE tissues and incubated at standard culture conditions for 18 to 24 hours to allow penetration into the living tissue. A positive control (e.g., chlorpromazine) and appropriate solvent controls are also applied topically to RhE tissues and tested in parallel. Half of the tissues in each treatment group are irradiated with 6 J/cm^2 of simulated sunlight (+Irr) while the remaining half are held at room temperature in the dark (−Irr). After a post-exposure incubation period of 18 to 24 hours, relative viability is determined in both the irradiated (+Irr) and non-irradiated (−Irr) treatment groups by measuring the enzymatic conversion of the vital dye MTT into a blue formazan salt that is measured photometrically after extraction from the tissues. Phototoxic potential can be estimated by comparing the relative reduction in viability in each irradiated treatment group to that of the equivalent non-irradiated treatment group.
Scientific basis including MoA	The test chemical is applied topically to a three-dimensional RhE tissue, composed of human-derived epidermal keratinocytes that have been cultured to form a multilayered, highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multilayered *stratum corneum* containing intercellular lamellar lipid layers representing main lipid classes analogous to those found *in vivo*. In comparison with monolayer culture system, the organic structure (multilayered and differentiated epidermis) and the presence of barrier function (*stratum corneum*) simulate more closely the *in vivo* situation and allow topical applications of a large panel of chemicals with different physiochemical properties.

Protocol available	OECD TG498
Strengths and weakness	Strengths - The RhE tissues can also tolerate UVB exposure, in comparison with monolayer culture system. - Wide variety of chemicals can be testable in RhE PT.- Can also be used to evaluate risk (e.g., NOEL/C) Weakness - In some countries, validated RhE tissue models are not available. - Quality control of the RhE model is required to meet defined production release criteria, among which those for viability, barrier function and morphology.
Applicability domain and limitations	Applicability - The test method is applicable to substances, complex mixture, and formulations. Limitations - Test chemicals with potent UV absorption in the same range as MTT formazan, or test chemicals able to directly reduce the vital dye MTT may interfere with the cell viability measurements (however can be addressed using specific controls described in the TG).
Predictive capacity	An initial test method pre-validation reported in 1999 with a sensitivity of 86.7% and specificity of 93.3% (set of 10 chemicals tested twice independently in three laboratories). Assay performance of RhE PT was further supported by follow-up studies.
Reliability	The reliability and relevance of the in vitro RhE PT was evaluated in multiple studies.

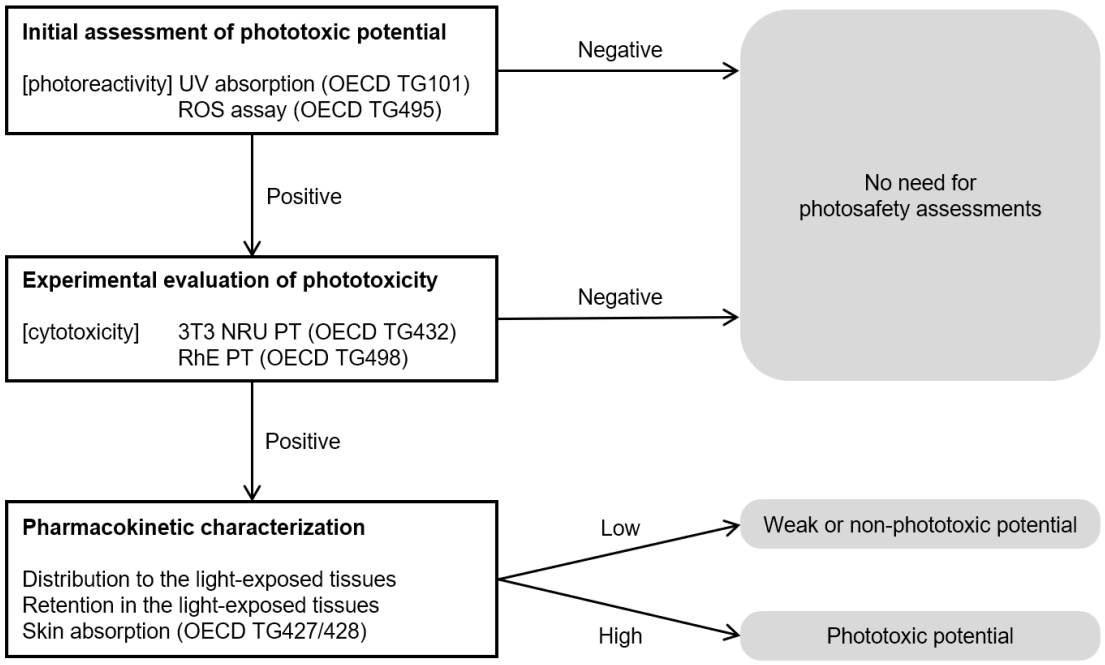

Figure 2: An example of integrated photosafety testing approach. In the 3T3 NRU PT, 'equivocal phototoxicity' prediction should be treated as positive.

また、これらの information sources を組み合わせた包括的光安全性評価に関して decision tree を新たに提案し、複数の評価系を組み合わせた光安全性評価の一例として IATA に記述した (Fig. 2)。

Decision tree は主に 3 つのカスケードによって構築され、(i) Initial assessment of phototoxic potential 、 (ii) experimental evaluation of phototoxicity 、 (iii) Pharmacokinetic characterization からなる。最初の光安全性評価は光化学的特性を指標としたものであり、主として UV/VIS 吸収特性や ROS assay から構築される。光安全性評価の初期段階でこれら光化学的特性を明らかにすることは ICH S10 とも矛盾せず、高いスループットによって迅速に光毒性リスクを示唆することができる。これらが陰性であった場合にはそれ以降は特に追加での光安全性評価を必要としないが、仮に光毒性リスクが疑われる結果であった場合にはフォローアップ試験として光生物学的アッセイ系によって更なる評価を行うことができる。この段階での光毒性リスク評価には 3T3 NRU PT あるいは RhE PT が用いられ、これらはより直接的な光毒性反応を示唆するものである。どちらの評価系を使用するかは特に規定しないが、被験物質の物理化学的特性や性状を考慮したうえで試験者が適切に選択することができる。このフォローアップ試験で陰性の場合は光安全性の懸念が特にないものとして判断することができる。一方、光毒性リスクが疑われる場合においては、更なるフォローアップ試験として薬物動態試験を実施し、経皮適用の場合には皮膚透過性・滞留性や蓄積性を、そして全身投

与の場合には皮膚移行性や皮膚滞留性等を精査することによって、実質的な光毒性リスクを検証することができる。ただし、体内動態に関する一定の閾値を設定することは難しく、被験物質毎に光反応性、光生物学的特性や投与量を考慮した上で適切な判断が求められ、さらに評価の妥当性については科学的合理性が許容される種々の実験データをもって評価者自身によって証明される必要がある。

D. 考察ならびに結論

信頼性の高い光安全性保障システム構築を指向して AOP ならびに IATA 作成に従事した。既に wiki に入力した AOP 案をさらに推敲し、光刺激性に関する毒性カスケードに焦点を当てたものに作り直し、外部評価に資するものに結実させた。この AOP をもとに IATA をアップデートするとともに、information sources に関する情報を多く加え、そして decision tree を新規に設定した。改訂した IATA 案はすでに専門機関内において commenting round に入ったので今後はコメントや指摘事項に対応して修正作業を行う予定である。

E. 研究発表

E-1. 論文発表

1. Ghosh A, Banik S, Suzuki Y, Mibe Y, Rikimura S, Komamoto T, Kuromi K, Yamada K, Sato H, Onoue S. Lysophosphatidylcholine-based liposome to improve oral absorption and nephroprotective effects of astaxanthin. *Journal of the Science of Food and Agriculture*, **103(6):** 2981–2988 (2023)
2. Ohtake H, Tokuyoshi Y, Iyama Y, Nukaga T, Nishida H, Ohtake T, Hirota M, Yamada K, Seto Y, Sato H, Kouzuki H, Onoue S. Reactive oxygen species (ROS) assay-based photosafety screening for complex ingredients: Modification of the ROS assay protocol. *Journal of Toxicological Sciences*, **47(11):** 483–492 (2022)
3. Nakamura K, Kambayashi A, Onoue S. Quantitative assessment of disintegration rate is important for predicting the oral absorption of solid dosage forms containing poorly soluble weak base drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, **180:** 23–32 (2022)
4. Halder S, Mibe Y, Rikimura S, Kuromi K, Sato H, Onoue S. Strategic application of liposomal system to R-α-lipoic acid for the improvement of nutraceutical properties. *Drug Development and Industrial Pharmacy*, **48:** 239–246 (2022)
5. Sato H, Yamane C, Higuchi K, Shindo T, Shikama H, Yamada K, Onoue S. Development of stabilized fuzapladib solution for injection: forced degradation study and pharmacokinetic evaluation. *Pharmaceutical Development and Technology*, **27(5):** 565–571 (2022)
6. Banik S, Yamada K, Sato H, Onoue S. Development of poly(lipoic acid) nanoparticles with improved oral bioavailability and hepatoprotective effects of quercetin. *Molecular Pharmaceutics*, **19(5):** 1468–1476 (2022)
7. Yamada K, Hayashi Y, Sasaki K, Higuchi K, Shindo T, Shikama H, Sato H, Onoue S. Nanocrystal solid dispersion of fuzapladib free acid with improved oral bioavailability. *Biopharmaceutics&Drug Disposition*, **43(3):** 89–97 (2022)
8. Yamada S, Niiya R, Ito Y, Kato Y, Onoue S. Comparative characterization of β-adrenoceptors in the bladder, heart, and lungs of rats: Alterations in spontaneously hypertensive rats. *Journal of*

Pharmacological Sciences, **148(1):** 51–55 (2022)

E-2 学会発表

1. 増井一樹，山田幸平，佐藤秀行，尾上誠良「経皮適用化合物の光安全性評価：被験物質の光反応性と *in vitro* 皮膚内曝露の統合的解析」日本薬学会第 142 年会（名古屋），2022 年 3 月 26 日
2. 桑原菜摘，袴田雅俊，淺沼俊倫，山下里恵 ，山田幸平，佐藤秀行，尾上誠良「白びわ抽出物由来の機能性化粧品素材開発 －カフェ酸誘導体の光保護作用評価－」第 47 回日本香粧品学会 (東京)，2022 年 6 月 10 日
3. 尾上誠良「薬剤性光線過敏症のリスク回避に向けた国際ガイドラインの作成」第 8 回日本医薬品安全性学会学術大会 (オンライン), 2022 年 8 月 20–9 月 4 日
4. Satomi Onoue “Establishment and International Harmonization of Photosafety Testing Strategy (国際シンポジウム)” 日本動物実験代替法学会第 35 回大会 (静岡), 2022 年 11 月 18 日
5. 山田幸平，上林敦，佐藤秀行，尾上誠良「*In silico* modeling & simulation による吸入毒性試験効率化を目指して」日本動物実験代替法学会第 35 回大会 (静岡)，2022 年 11 月 19 日

F. 知的財産権の出願・登録状況

該当なし