Toxicology in Vitro 33 (2016) 147–152

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Comparative study on prediction performance of photosafety testing tools on photoallergens

Satomi Onoue [a,*], Hiroto Ohtake [a], Gen Suzuki [a], Yoshiki Seto [a], Hayato Nishida [b], Morihiko Hirota [b], Takao Ashikaga [b], Hirokazu Kouzuki [b]

[a] Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[b] Shiseido Research Center, Shiseido Co. Ltd., 2-2-1 Hayabuchi, Tsuzuki-ku, Yokohama-shi, Kanagawa 226-8553, Japan

ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form 21 January 2016
Accepted 6 March 2016
Available online 10 March 2016

Keywords:
Photoallergenicity
Photosafety assessment
Phototoxicity
Reactive oxygen species
UV

ABSTRACT

Several testing methods have been established to identify potential phototoxins. The present study was undertaken to clarify the predictive ability of *in vitro* photosafety assays for photoallergenicity. On the basis of animal and/or clinical photosafety information, 23 photoallergens and 7 non-phototoxic/non-photoallergenic chemicals were selected and subjected to UV/VIS spectral analysis, reactive oxygen species (ROS)/micellar ROS (mROS) assays, and 3T3 neutral red uptake phototoxicity testing (3T3 NRU PT). Of the photoallergens tested, *ca.* 96% of chemicals had intense UV/VIS absorption with a molar extinction coefficient of over 1000 M^{-1} cm^{-1}, and false-positive predictions were made for 3 non-photoallergenic chemicals. In the ROS assay, all photoallergens were found to be potent ROS generators under exposure to simulated sunlight. In the photosafety prediction based on the ROS assay, the individual specificity was 85.7%, and the positive predictivity and negative predictivity were found to be 95.8% and 100%, respectively. Most of the photoirritant chemicals were correctly identified by the 3T3 NRU PT; however, it provided false predictions for *ca.* 48% of photoallergens. The orders of sensitivity and specificity for photoallergenicity prediction were estimated to be: [sensitivity] ROS assay > UV/VIS absorption ≫ 3T3 NRU PT, and [specificity] 3T3 NRU PT > ROS assay ≫ UV/VIS absorption. Thus, photochemical assays, in particular the ROS assay, can be used for assessment of photoallergenicity, although there were some false-positive predictions.

1. Introduction

Phototoxic responses in light-exposed tissues can be caused by several classes of pharmaceuticals, cosmetics and foods, and these phototoxic events can be categorized into photoirritation, photoallergy and photogenotoxicity in accordance with their mechanisms and outcomes (Moore, 1998, 2002). Concerns about phototoxicity and its avoidance are growing, and a number of *in vitro* assay systems have been developed for photosafety assessment over the past few years (Onoue et al., 2009). The International Conference on Harmonization (ICH) S10 guidelines on photosafety evaluation successfully reached step 5 of the ICH process in 2014, describing detailed photosafety assessment strategies on the basis of photochemical and photobiochemical properties, and *in vivo* pharmacokinetic behavior (ICH, 2014). In the ICH S10 guideline, three *in vitro* photosafety test methods are recommended: (i) UV spectral analysis (Henry et al., 2009), (ii) reactive oxygen species (ROS) assay (Onoue and Tsuda, 2006), and (iii) 3T3 neutral red uptake phototoxicity test (3T3 NRU PT) (Spielmann et al., 1994).

Absorption of sunlight by phototoxins, followed by photochemical reaction, is considered to be a key trigger for phototoxicity (Onoue et al., 2013b), because photo-excited chemicals may react with biomolecules, leading to phototoxic events (Moore, 1998, 2002). In this context, the UV-absorbing property of chemicals can be a potential indicator for phototoxic risk, and Henry and co-workers demonstrated that chemicals with a molar extinction coefficient (MEC) of less than 1000 M^{-1} cm^{-1} showed low phototoxic risk (Henry et al., 2009). Photo-excited chemicals tend to generate ROS, resulting in oxidative damage to the cellular membrane, DNA and other biomolecules (Brendler-Schwaab et al., 2004; Epstein and Wintroub, 1985); therefore, the ROS assay of photoirradiated chemicals has been used for photosafety assessment (Onoue et al., 2014). On the other hand, 3T3 NRU PT was originally established to assess the cytotoxicity of

Abbreviations: 3T3 NRU PT, 3T3 neutral red uptake phototoxicity testing; AOP, Adverse Outcome Pathway; DMSO, dimethyl sulfoxide; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; IFRA, International Fragrance Association; JaCVAM, Japanese Center for the Validation of Alternative Methods; JPMA, Japan Pharmaceutical Manufacturers Association; MEC, molar extinction coefficient; mROS assay, micellar ROS assay; NaPB, sodium phosphate buffer; NBT, nitroblue tetrazolium; OECD, Organisation for Economic Co-operation and Development; PIF, photoirritation factor; ROS, reactive oxygen species; UV, ultraviolet; VIS light, visible light.

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

http://dx.doi.org/10.1016/j.tiv.2016.03.003
0887-2333/

photo-excited chemicals as an *in vitro* alternative to *in vivo* phototoxicity tests (Liebsch and Spielmann, 2002).

Photoirritation is a narrowly specified type of phototoxicity that can be defined as an inflammatory event in UV-exposed tissues, triggered by photo-oxidation of lipids and proteins in the cellular membrane (Girotti, 2001; Schothorst et al., 1972), while photoallergy is an immune response to photosensitizer-bound proteins (Tokura, 2009). The *in vitro* assays recommended in the ICH S10 guideline are well validated and have a high predictive capacity for photoirritancy of tested chemicals (ICH, 2014). Although a previous study demonstrated that the photoallergenic potential of tested chemicals might be partly identified by ROS assay (Onoue et al., 2013c), the applicability of these *in vitro* assessments for predicting photoallergenic risk is still poorly understood. Therefore, we undertook the present study to clarify the predictive performance of photochemical and photobiochemical assays for the photoallergenic potential, using a panel of 23 photoallergens and 7 non-phototoxic/non-photoallergenic chemicals (Table 1). These model chemicals were assessed by means of UV/VIS spectral analysis, ROS assay, and 3T3 NRU PT, and the results were compared.

2. Materials and methods

2.1. Chemicals

On the basis of published photosafety data and the International Fragrance Association (IFRA) standard (Bakkum and Heule, 2002; Hindsen et al., 2006; Horio et al., 1994; Kerr et al., 2010; Lovell, 1993; Lugovic et al., 2007; Moore, 2002; Murata et al., 1998; Onoue et al., 2013c; Scheinfeld et al., 2014; Tokura, 2009), 30 chemicals, including 23 photoallergens and 7 non-phototoxic chemicals, were selected for the present study (Table 1). Dimethyl sulfoxide (DMSO), erythromycin (**27**), glibenclamide (**8**), hexachlorophene (**9**), indomethacin (**11**), imidazole, ketoprofen (**13**), 8-methoxypsoralen (**2**), 4′-methylbenzylidene camphor (**24**), nitroblue tetrazolium (NBT), *p*-nitrosodimethylaniline, phenytoin (**30**), piroxicam (**18**), sulfanilamide (**20**) and tribromsalan (**22**) were bought from Wako Pure Chemical Industries (Osaka, Japan). Aspirin (**25**), bithionol (**4**), dichlorophene (**5**), enoxacin (**6**), octyl dimethyl PABA (**16**), penicillin G (**29**), pyridoxine HCl (**19**) and triclocarban (**23**) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Benzophenone (**3**), hydrochlorothiazide (**10**) and methylsalicylate (**28**) were obtained from Junsei Chemical Co. (Tokyo, Japan), and 6-methylcoumarin (**1**) was purchased from Nacalai Tesque (Kyoto, Japan). Benzocaine (**26**), fenticlor (**7**), musk ambrette (**14**) and musk xylene (**15**) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Isoniazid (**12**) was obtained from LKT Laboratories (St. Paul, MN, USA). Omadine Na (**17**) was bought from Alfa Aesar (Heysham, UK). Sulfasalazine (**21**) was purchased from Fluka (St. Gallen, Switzerland). A quartz reaction container for high-throughput ROS assay (Onoue et al., 2008a) was constructed by Ozawa Science (Aichi, Japan).

2.2. UV/VIS spectral analysis

Each chemical was dissolved in methanol or distilled water at final concentrations of 0.001, 0.01 and 0.1 μM, and the final concentration was reduced if the tested chemical was found to be an intense UV/VIS absorber. UV/VIS absorption spectra were recorded with a UV–VIS Multipurpose Spectrophotometer MPS-2400 (Shimadzu Corporation, Kyoto, Japan) interfaced to a PC for data processing (software: UVProve Version 1.12). MEC values were determined from absorbance values for peaks tailing through 290 nm from a previous maximum absorbance, and all peaks were detected at 290 nm or higher wavelength.

Table 1
Test chemicals.

No.	Chemical name	CAS number	Physical state	Color	Molecular weight	Clog P[b]	Category[c]
Photoallergens							
1	6-Methylcoumarin	92-48-8	Powder	White	160.05	1.91	C
2	8-Methoxypsoralen	298-81-7	Powder	Light yellow	216.04	2.31	C, P
3	Benzophenone	119-61-9	Powder	White	182.07	3.18	C
4	Bithionol	97-18-7	Powder	White	353.88	6.16	C
5	Dichlorophene	97-23-4	Powder	Light yellow	268.01	4.79	P
6	Enoxacin	74011-58-8	Powder	White	320.13	−1.60	P
7	Fenticlor	97-24-5	Powder	Light yellow	285.96	5.19	C
8	Glibenclamide	10238-21-8	Powder	White	493.14	4.24	P
9	Hexachlorophene	70-30-4	Powder	White	403.85	7.03	C
10	Hydrochlorothiazide	58-93-5	Powder	White	296.96	−0.36	P
11	Indomethacin	53-86-1	Powder	Yellow	357.08	4.18	P
12	Isoniazid	54-85-3	Powder	White	137.06	−0.67	P
13	Ketoprofen	22071-15-4	Powder	White	254.09	2.76	P
14	Musk ambrette	83-66-9	Powder	Yellow	268.11	3.84	C
15	Musk xylene	81-15-2	Powder	White	297.10	3.96	C
16	Octyl dimethyl PABA	21245-02-3	Liquid	Light yellow	277.20	6.16	C
17	Omadine Na	3811-73-2	Powder	White	150.00 (127.01)[a]	−0.59	C
18	Piroxicam	36322-90-4	Powder	White	331.06	1.89	P
19	Pyridoxine HCl	58-56-0	Powder	White	205.05 (169.07)[a]	−0.35	P, F
20	Sulfanilamide	63-74-1	Powder	White	172.03	−0.57	P
21	Sulfasalazine	599-79-1	Powder	Yellow	398.07	3.99	P
22	Tribromsalan	87-10-5	Powder	White	446.81	6.01	P (animal)
23	Triclocarban	101-20-2	Powder	White	313.98	5.47	C
Non-phototoxic/non-photoallergic chemicals							
24	4′-Methylbenzylidene camphor	36861-47-9	Powder	White	254.17	5.02	C
25	Aspirin	50-78-2	Powder	White	180.04	1.02	P
26	Benzocaine	94-09-7	Powder	White	165.08	1.92	P
27	Erythromycin	114-07-8	Powder	White	791.47	1.66	P
28	Methylsalicylate	119-36-8	Liquid	Yellow	152.05	2.33	P
29	Penicillin G	113-98-4	Powder	White	387.08 (348.11) [a]	2.27	P
30	Phenytoin	57-41-0	Powder	White	252.09	2.09	P

[a] Number in parenthesis represents molecular weight of free compound.
[b] Calculated on ChemBioDraw Ultra 13.0 software.
[c] C, cosmetic ingredients; F, food ingredients; and P, pharmaceutics.