

Introduction

Nonclinical photosafety evaluation is required for drug candidates that absorb light within the range of natural sunlight (290-700 nm) and have bio-distributions to light-exposed tissues. A stepwise approach involving chemical assay, in vitro assay, and in vivo assay is recommended for testing of phototoxicity potential. Although some in vitro assays have been validated, there are limitations including high false positive rate, compound solubility issue, or limited use for topical application drugs. The in vivo phototoxicity assay is needed when the in vitro study reports a positive result. While a variety of species have been used for in vivo phototoxicity study, no standardized study design has been established to date. The purpose of this investigation is to determine the phototoxicity of 8-MOP in rats and guinea pigs when administered orally, using a sunlight simulator light source endorsed by the in vitro 3T3 NRU phototoxicity test guideline.

Methods

Five groups of 3 female Hartley guinea pigs/group were administered corn oil or 5, 10, and 15 mg/kg/day 8-MOP once daily for 3 days by oral gavage. All guinea pigs were irradiated at 10 J/cm² UVA by a solar simulator (SOL500, Dr. Hönle AG) or sham irradiated (15 mg/kg/day only) following the last dose. Six groups of 3 female Long-Evans rats/group were administered corn oil or 5, 15, and 50 mg/kg/day 8-MOP once daily for 3 days by oral gavage. All rats were irradiated at 10 J/cm² UVA (all dose levels) and at 5 J/cm² UVA (50 mg/kg/day only), or sham irradiated (50 mg/kg/day only) following the last dose. The exposed dermal skins were scored (edema and erythema/scar) up to 72 hours post irradiation. Ophthalmic examinations were performed on the last day of in life. Skin and eye tissues were collected for histopathology evaluation.

Results

In both species, oral administration of 8-MOP followed by irradiation resulted in dose related changes of phototoxicity in the skin and eyes of test animals. Skin edema and erythema (Tables 1 and 2), periorbital redness/swelling and corneal opacity were noted during clinical observations; cornea edema and/or dystrophy, conjunctiva hyperemia, fundus unclear, iris hyperemia, aqueous flare, ectropion, eyelid swelling and/or redness, intraocular structure not visible were noted at ophthalmic examinations; and at pathology evaluation, cell necrosis, hypertrophy or hyperplasia, ulceration, and crust were noted in the epidermis, congestion, hemorrhage, mononuclear infiltration, fibroplasia, and edema were noted in the dermis, and corneal epithelial necrosis, neutrophilic infiltration and/or edema of the cornea, and epithelial degeneration/necrosis of the lens were noted in the eyes (Tables 3 and 4). In the rat study, phototoxicity reactions were not meaningfully different between pigmented/non-pigmented skins, or between animals dosed at 50 mg/kg/day 8 MOP and irradiated at 10 and 5 J/cm² UVA.

Table 1 Summary of Dermal Scores – Guinea Pigs

8-MOP Dose (mg/kg/day)	UVA Dose (J/cm ²)	Time Points Relative to Irradiation					
		Pre-dose	1 h	4 h	24 h	48 h	72 h
0	10	0	0	0	0	0	0
5	10	0	0	0	0	0	0
10	10	0	0	0	1-4	1-5	2-5
15	10	0	0	0	5	4-6	4-6
15	0	0	0	0	0	0	0

Table 3 Summary of Histopathology Findings – Guinea Pigs

8-MOP Dose (mg/kg/day)	0	5	10	15	15
UVA Dose (J/cm ²)	10	10	10	10	0
Number of Animals	3	3	3	3	3
Skin					
Necrosis, epidermis, squamous, minimal	0	3	2	0	0
Hypertrophy and/or hyperkeratosis, squamous, epidermis, minimal to mild	0	0	3	0	0
Degeneration/necrosis, squamous, epidermis, moderate	0	0	1	0	0
Necrosis, epidermis, dermis, minimal to marked	0	0	1	3	0
Edema, dermis, hypodermis, minimal	0	0	1	0	0
Congestion/hemorrhage, dermis, hypodermis, and/or subcutis, minimal to mild	0	0	1	3	0
Fibroplasia, dermis, hypodermis, and/or subcutis, minimal to mild	0	0	1	3	0
Neovascularization, dermis, hypodermis, and/or subcutis, minimal	0	0	1	3	0
Infiltration, mononuclear, dermis, hypodermis, and/or subcutis, minimal to mild	0	0	3	3	0
Infiltration, neutrophilic, epidermis, mild	0	0	0	1	0
Eyes					
Necrosis, epithelial, cornea, minimal	0	0	0	1	0
Edema, cornea, mild	0	0	0	1	0
Infiltration, neutrophilic, cornea, and/or ciliary body, minimal to mild	0	0	1	3	0
Degeneration/necrosis, epithelial, lens, minimal	0	0	0	1	0

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References

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2. Assessment of 8-Methosypsoralen, Lomefloxacin, Sparfloxacin, and Pirfenidone phototoxicity in Long-Evans Rats, Tamiko Adachi et al. Int J Toxicol. 2015 Jan-Feb. 34(1):16-23.

Table 2 Summary of Dermal Scores – Rats

8-MOP Dose (mg/kg/day)	UVA Dose (J/cm ²)	Time Points Relative to Irradiation - Pigmented/Non-Pigmented Skin					
		Pre-dose	1 h	4 h	24 h	48 h	72 h
0	10	0	0	0	0	0	0
5	10	0	0	0	0	1-2/1-2	1-3/2-4
15	10	0	0	0	1/0-3	4-7/6-7	7/7
50	10	0	0	0	0-2/1	4-7/6-7	6-7/7
50	5	0	0	0	0-1/0-1	4-6/6-7	5-7/6-7
50	0	0	0	0	0	0	0

Table 4 Summary of Histopathology Findings – Rats

8-MOP Dose (mg/kg/day)	0	5	15	50	50	50
UVA Dose (J/cm ²)	10	10	10	10	5	0
Number of Animals	3	3	3	3	3	3
Skin (Non-Pigmented)						
Ulceration, epidermis, minimal to marked	0	1	3	3	3	0
Crust, mild to marked	0	0	3	2	3	0
Hyperplasia, epidermis, minimal to mild	0	3	3	3	2	0
Necrosis, single cell, epidermis, minimal	0	3	0	0	0	0
Congestion/Hemorrhage, dermis, minimal to mild	0	2	3	3	3	0
Infiltration, mononuclear, dermis, minimal to mild	0	1	3	3	3	0
Fibroplasia, dermis, minimal to mild	0	0	2	3	3	0
Skin (Pigmented)						
Ulceration, epidermis, moderate to marked	0	0	3	3	3	0
Crust, mild to marked	0	0	3	2	2	0
Hyperplasia, epidermis, minimal to mild	0	3	3	3	3	0
Necrosis, single cell, epidermis, minimal	0	3	0	0	0	0
Congestion/Hemorrhage, dermis, minimal to mild	0	1	3	3	3	0
Infiltration, mononuclear, dermis, minimal to mild	0	1	3	3	3	0
Fibroplasia, dermis, minimal to mild	0	0	3	3	3	0
Eyes						
Degeneration/Necrosis, epithelial, cornea, minimal to moderate	0	3	3	3	3	0
Degeneration/Necrosis, epithelial, lens, minimal	0	1	3	1	0	0
Infiltration, neutrophilic, cornea, minimal to mild	0	0	3	3	3	0
Edema, cornea, minimal to mild	0	0	3	3	3	0
Ulceration, cornea, minimal	0	0	0	1	0	0