Development of a Phototoxicity Testing Strategy for Accurate Photosafety Evaluation of Pharmaceuticals based on the Assessment of Possible Melanin Binding Effects

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1 BACKGROUND

Drug-induced phototoxicity is an undesirable reaction which is the effect of an interaction of a photoactivated drug with biomolecules present in the skin and eyes. Drug-induced phototoxicity occurs when a topically or systemically administered drug is excited by absorption of photon energy after exposure to natural sunlight. Therefore the distribution to light exposed tissues, such as the skin and eyes, is relevant for photoactivation of drugs. Although binding, retention or accumulation of a photoreactive drug in tissues is not essential for a reaction to occur, longer mean residence times in light exposed tissues do increase the likelihood for the photoreactive drug to produce an adverse phototoxic reaction.

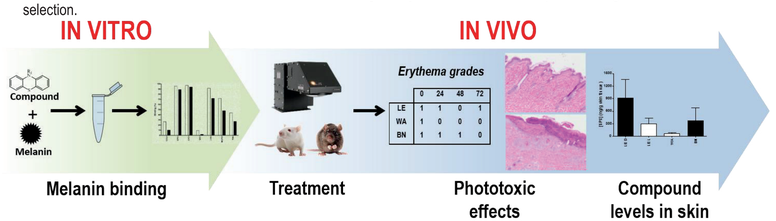
One mechanism by which a photoreactive drug can have longer residence times or accumulate in tissues is by binding to the naturally occurring biopolymer melanin. In humans, melanin is present in many tissues including the eye, skin and hair. Various drugs, including antimalarial, antibiotics, beta blockers, CNS drugs and anticholinergics, have been reported to bind to melanin both *in vitro* and *in vivo*. Since turnover of melanin in the body is low, a long-term retention in melanin-containing tissues of drugs with high melanin affinity may occur. As melanin-rich areas of the body are extensively exposed to light, binding of phototoxic drugs to melanin potentially increases their phototoxicity.

For in vivo photosafety studies, models with both pigmented and non-pigmented animals are available. The International Council on Harmonization (ICH) issued a guidance document (S10) which states that: *"although non-pigmented animals are generally more sensitive to light for detecting phototoxicity, a pigmented model should be considered if a compound has a high affinity to melanin"*. Therefore it is important select the appropriate model based on the melanin binding affinity of photoreactive drugs, in order to achieve accurate risk assessment.

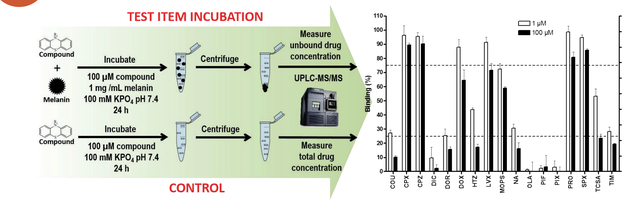
2 STUDY OBJECTIVES

The goal of this study was to develop a testing strategy which includes the assessment of melanin binding affinities for accurate photosafety evaluation.

In the current study, we have applied an in vitro melanin binding screening assay to study the binding of various structurally diverse phototoxic and/or melanin binding drugs to synthetic melanin in vitro. From this screening experiment we have selected the high affinity binder sparfloxacin (SPX), moderate affinity binders 8-methoxypsoralen (MOPS) and nalidixic acid (NA) and low affinity binder pirfenidone (PIF) for which we assessed the phototoxic effects in different in vivo models. These drugs with varying melanin affinities were tested in a pigmented model (Brown Norway Rat) and a non-pigmented model (Wistar Albino Rat). It was evaluated whether the in vitro melanin binding assay could be used as a prescreening tool for animal model selection.



3 IN VITRO RESULTS – COMPOUND SELECTION



Melanin binding affinity of a structurally diverse set of chemicals was screened at two substrate concentrations (1 and 100 µM) in order to identify high (>75% binding at 1 µM), moderate (25–75% at 1 µM) and low (<25% at 1 µM) affinity binders.

High affinity binders

Ciprofloxacin (CPX)
Chlorpromazine (CPZ)
Doxycycline (DOX)
Levofloxacin (LVX)
Procaine (PRO)

Sparfloxacin (SPX) - 95%

Moderate affinity binders

Coumarin (COU)
Dorzolamide (DOR)
Hydrochlorothiazide (HTZ)
Tetrachlorosalicylanilide (TCSA)
Timolol (TIM)

8-Methoxypsoralen (MOPS) - 72%
Nalidixic acid (NA) - 31%

Low affinity binders

Diclofenac (DIC)
Olaquindox (OLA)
Doxycycline (DOX)
Piroxicam (PIX)

Pirfenidone (PIF) - 2%

4 IN VIVO RESULTS

Dosing
- Vehicle (1% carboxymethyl cellulose) or phototoxic compound dosed by oral gavage for three consecutive days

Irradiation
- Single irradiation after last dose using a Newport Oriel Solar Simulator
- Sunlight spectrum with UVB filter
- Dose of 12 J/cm² UVA

Observations
- Erythema assessment before and 0, 0.5, 1, 4, 24, 48 and 72 hours after the end of irradiation
- Histopathology of the skin after the last observation

Analysis
- Excision of skin samples after necropsy
- Processing of skin samples
- Analysis of compound concentrations in skin extracts

Assessment of skin reactions

Compound	Rat strain	Before	Time after irradiation (hours) 0	0.5	1	4	24	48	72
SPX 100 mg/kg	WA	0	0	0	0	1	1	2	1
	BN	0	1	1	1	2	1	2	2
MOPS 15 mg/kg	WA	0	1	0	0	1	1	1	2
	BN	0	0	0	0	1	1	1	2
NA 400 mg/kg	WA	0	1	1	1	1	-	-	-
	BN	0	1	0	0	0	-	-	-
PIF 300 mg/kg	WA	0	0	0	0	0	0	1	0
	BN	0	0	0	0	0	0	0	0
PIF 600 mg/kg	WA	0	0	0	0	0	0	0	0
	BN	0	0	0	0	0	0	0	0

Table 1 Treatment related skin reactions observed for Wistar Albino (WA) and Brown Norway (BN) rats. These scores were calculated as the highest score of the irradiated drug dosed group minus the highest score of control groups (non-irradiated drug dosed and irradiated vehicle dosed groups). No erythema was observed for the animals of the control groups. 0 = no erythema, 1 = very slight erythema, 2 = well-defined erythema, - = not scored

Histopathology

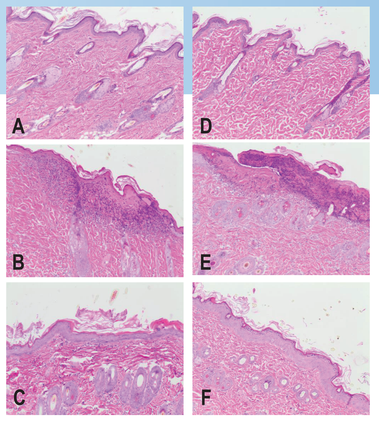


Figure 1 Histopathology of the skin of the (A) irradiated control Wistar Albino (WA) showed no findings. (B) MOPS dosed irradiated WA showed moderate inflammatory infiltrate in the dermis and moderate epidermal ulceration. (C)SPX dosed irradiated WA showed minimal inflammatory infiltrate in the dermis and slight epidermal hyperplasia. (D) Irradiated control Brown Norway (BN) showed no findings. (F) MOPS dosed irradiated BN showed moderate inflammatory infiltrate in the dermis and massive epidermal ulceration. (E) SPX irradiated dosed BN showed moderate epidermal hyperplasia. NA and PIF showed no histopathological findings.

Compound levels in skin

Compound	Rat strain	Mean ± SEM	Range	# Animals
SPX 100 mg/kg	WA	115 ± 16	46 – 197	9
	BN	1204 ± 440	75 – 4029	9
MOPS 15 mg/kg	WA	9 ± 1	6 – 13	6
	BN	9 ± 2	4 – 20	6
NA 400 mg/kg	WA	17 ± 2	14 – 21	3
	BN	20 ± 5	10 – 41	6
PIF 300 mg/kg	WA	92 ± 10	58 – 117	6
	BN	66 ± 13	31 – 112	6
PIF 600 mg/kg	WA	28 ± 9	11 – 72	6
	BN	63 ± 42	18 – 148	3

Table 2 Test compound concentrations measured in skin samples of Wistar Albino (WA) and Brown Norway (BN) rats. For MOPS, PIF and SPX the concentrations are given in ng/g skin whereas for NA concentrations are given in µg/g skin. SEM = Standard error of the mean.

5 CONCLUSION

- The present study showed that a combined in vitro/in vivo approach improves accurate photosafety evaluation of pharmaceuticals.
- It was shown that variation in melanin binding affinity causes differences in severity of the phototoxic skin reactions between the pigmented and non-pigmented animal models. The pigmented model showed higher sensitivity for drugs with higher melanin affinity while drugs with lower affinity showed higher sensitivity in the non-pigmented model. Histopathology confirmed the erythema scores.
- For the high affinity melanin binder SPX, a significantly higher concentration was measured in the skin of the pigmented BN animals. This correlates with the severity of the skin reactions and confirms the predictive value of the in vitro melanin binding assay.
- Based on the current results, we would consider the pigmented model for compounds showing an in vitro melanin binding capacity exceeding 75%.