An *in vitro* melanin binding assay as prescreening tool for animal model selection for phototoxicity studies

Martine Kruijssen, Jelle Reinen, Ton van Huygevoort, Kevin Scase, Mira Wenker

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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 light. 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.

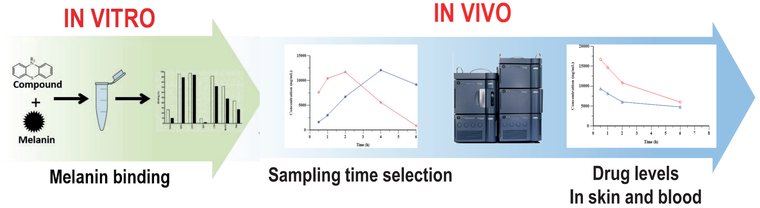
One mechanism by which a photoreactive drug can have longer residence times or accumulate in tissues is by binding to the naturally occurring biopolymer melanin. In humans, melanin is present in many tissues including the eye, skin and hair. Various drugs, including antimalarial, antibiotics, beta blockers, 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 to be able to 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 determine whether an *in vitro* melanin binding assay is predictable for the exposure of phototoxic compounds in the skin.

An *in vitro* melanin binding assay was performed to study the melanin binding capacity of phototoxic drugs to synthetic melanin. Based on the obtained results 8-methoxypsoralen (8-MOPS), lomefloxacin (LMX), nalidixic acid (NA), sparfloxacin (SPX) and pirfenidone (PIF), for which varying melanin binding capacities were determined, were selected for an *in vivo* pharmacokinetic study. In this study, the pharmacokinetics of the selected phototoxic drugs were determined in the blood and skin of a pigmented and a non-pigmented mouse model after three consecutive days of oral exposure. It was assessed whether accumulation of phototoxic compounds with high melanin binding affinities was higher in pigmented skin when compared to non-pigmented skin.



4 CONCLUSIONS

- For the *in vitro* high affinity binders, the skin/blood ratio for the AUC_{last} was approximately 2 times higher in pigmented mice for 8-MOPS and SPX and approximately 5 times higher in pigmented mice for LMX when compared to non-pigmented mice.
- The skin/blood ratio was similar for the *in vitro* low affinity binders NA and PIF in pigmented and non-pigmented mice.
- These results demonstrate that the exposure to compounds with high affinity to melanin in skin compared to blood is higher in pigmented mice compared to non-pigmented mice.
- A trend is seen between the results of the *in vitro* melanin binding assay and the results found during the *in vivo* pharmacokinetic study. This demonstrates that the *in vitro* melanin binding assay might be predictive for the distribution of drugs to melanin-rich tissues and might be a useful tool for the selection of the appropriate animal model for phototoxicity studies for systemic drugs.
- Future perspectives include assessment of phototoxic skin reactions in pigmented and non-pigmented animals of the selected drugs.
- The present study showed that a combined *in vitro/in vivo* approach improves animal selection for accurate photosafety evaluation of pharmaceuticals.

3 RESULTS

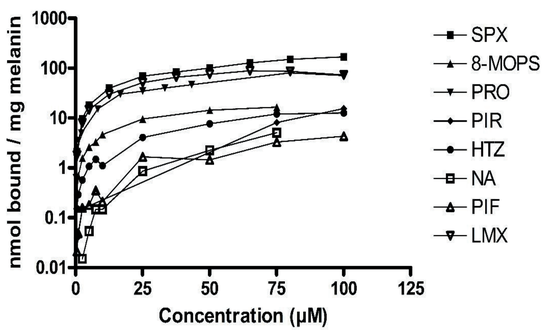


Figure 1. In vitro melanin binding results - Binding of compound per mg synthetic melanin. SPX = sparfloxacin, 8-MOPS = 8-methoxypsoralen, PRO = procaine, PIR = piroxicam, HTZ = hydrochlorothiazide, NA = nalidixic acid, PIF = pirfenidone, LMX = lomefloxacin

COMPOUND SELECTION

	IN VITRO BINDING	IN VIVO PHARMACOKINETICS				ASSESSMENT		
	B_{max} (nmol/mg melanin)	AUC_{last} Blood (h*nM)		AUC_{last} Skin (h*nM)		Observed ratio of AUC_{last} Skin / AUC_{last} Blood		Ratio Pigmented / Non-pigmented
		Pigmented	Non-pigmented	Pigmented	Non-pigmented	Pigmented	Non-pigmented	
Sparfloxacin	375.45	302000	829000	22500	32800	0.07	0.04	1.9
Lomefloxacin	111.15	92100	167000	19400	5980	0.21	0.04	5.9
8-Methoxypsoralen	27.38	60100	48700	13900	5680	0.23	0.12	2.0
Nalidixic acid	10.87	2090000	2800000	35300	57300	0.02	0.02	0.8
Pirfenidone	Not detectable	201000	221000	10600	13200	0.05	0.06	0.9

Table 2. Maximum binding capacity of phototoxic compounds to synthetic melanin and skin/blood ratios of known phototoxic compounds in pigmented DBA/2 and non-pigmented SKH1 mice following oral administration for three consecutive days.