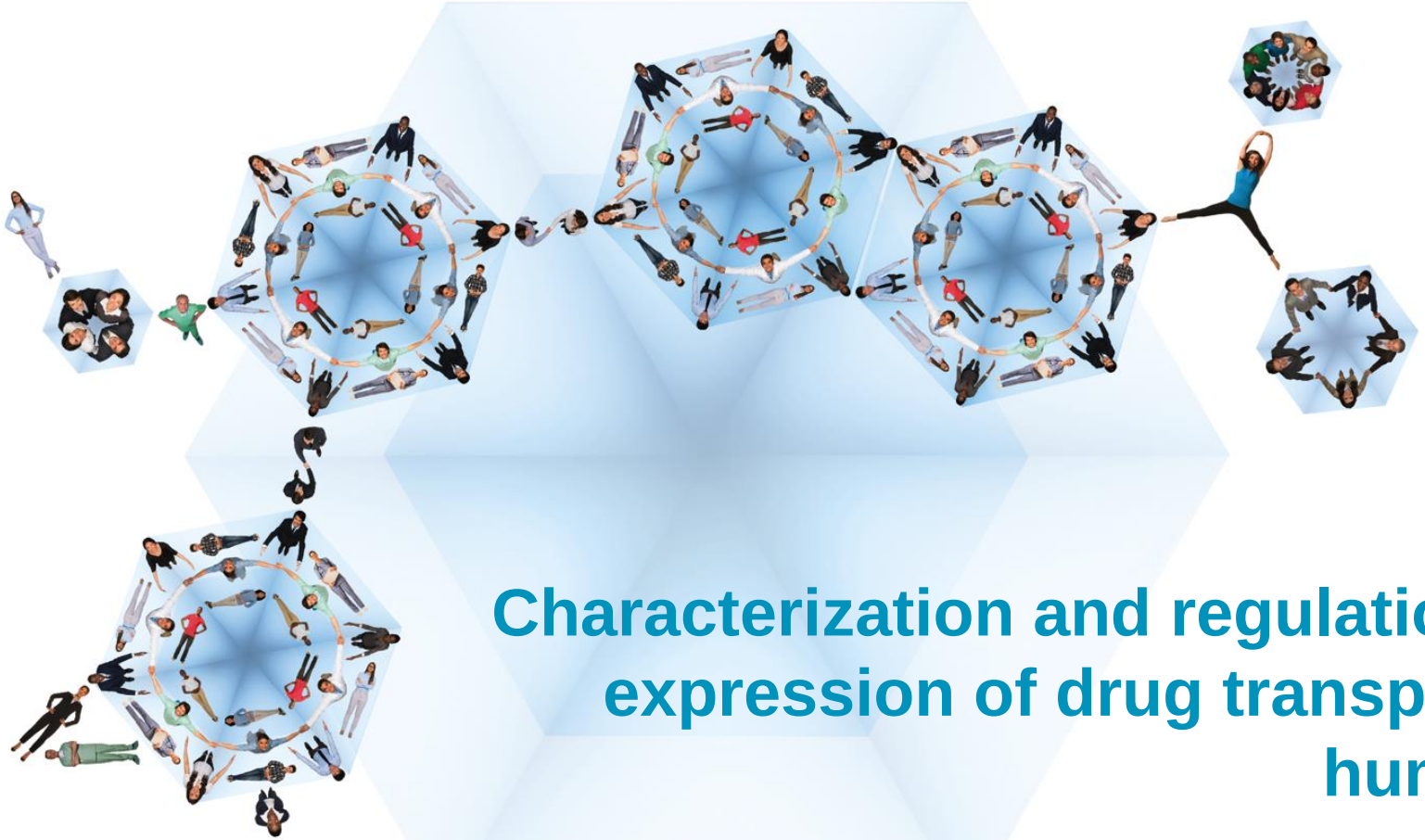

Characterization and regulation of the expression of drug transporters in human skin

Hanan Osman-Ponchet

Meet the Experts Transporter meeting
Budapest, May 11-13, 2016

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GALDERMA
Committed to the future of dermatology

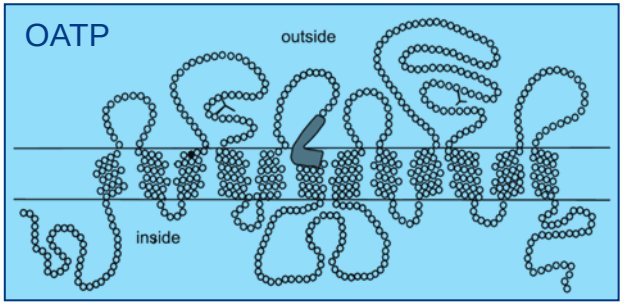
Outline

- General overview of drug transporters
  - ABC and SLC transporters
- Regulatory perspectives
- Characterization of drug transporters in human skin
  - Expression of ABC and SLC transporters
    - Comparison between skin, liver and kidney
  - Regulation of the expression by Rifampicin and UV irradiation
  - Subcellular localisation of MRP1 in human skin
  - Role of MRP1 transporter in drug uptake in human skin
- Conclusion

Drug transporters

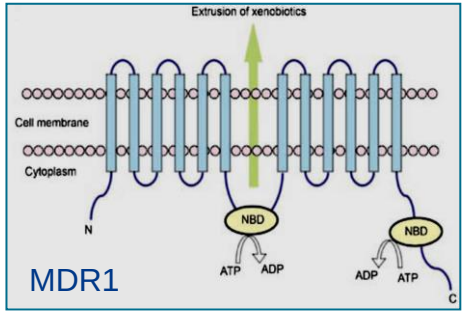
- Most identified drug transporters belong to 2 superfamilies:
  - ATP-Binding Cassette (ABC) → Drug Efflux (Out)
  - Solute Carrier (SLC) → Drug Uptake (In)
- Transmembrane proteins

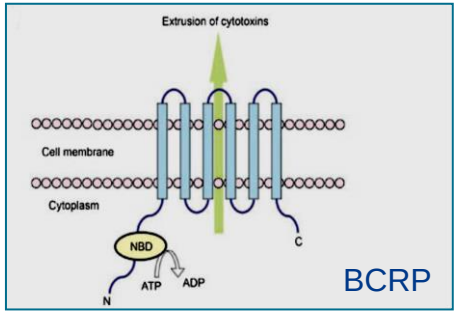
SLC



OATPs, S. Leuthold et al; Am J Physiol (2009)

ABC

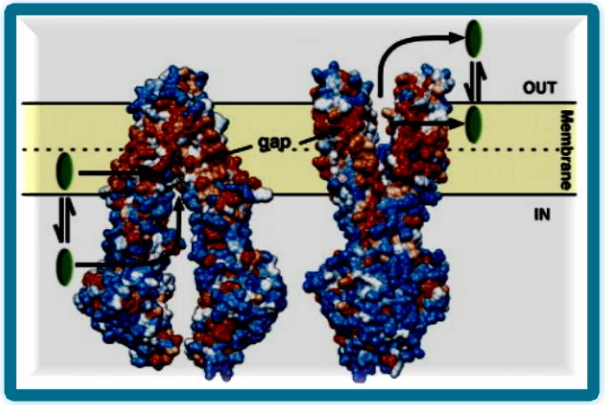




T. Lin et al; Cell Research (2006)

ABC transporter Superfamily

- Use energy of ATP hydrolysis to transport various substrates.



D. AP. Gutmann et al; Trend in Biochemical Sciences (2010).

EFFLUX

- 49 human ABC genes grouped into seven subfamilies:
  - ABCA, ABCB (MDR), ABCC (MRP)…., ABCG (BCRP)
- First mammalian ABC transporter cloned in 1986: P-glycoprotein (ABCB1)
- ABC transporters with multidrug transporter function:
  - ABCB1 / MDR1 (Multi-drug resistance)
  - ABCC1 & 2 / MRP1 & 2 (Multidrug resistance-associated protein)
  - ABCG2/ BCRP (Breast cancer resistance protein)

ABC transporters and genetic diseases

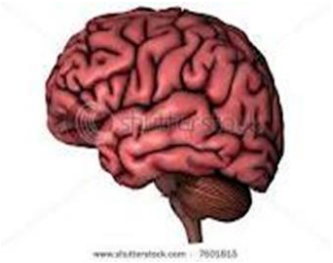
- In humans, 15 severe genetic diseases are caused by the dysfunction of ABC transporters:
  - ABCA1 : Tangier disease
  - ABCA12 : Harlequin-type ichthyosis, Lamellar ichthyosis
  - ABCB4 : Progressive familial intrahepatic cholestasis
  - ABCC2 : Dubin–Johnson syndrome
  - ABCC6 : Pseudoxanthoma elasticum
  - ABCC7 : Cystic fibrosis
- Genetic polymorphisms (SNP, haplotypes) identified, but their clinically relevance in drug pharmacokinetics not clearly demonstrated

ABC transporters – Localisation/tissue distribution

- All multidrug transporters are localized predominantly in the plasma membrane providing a cellular defense mechanism throughout the organism.

- ABCB1: tissues involved in the absorption and secretion and with pharmacological barrier function (blood-brain barrier)

- ABCC1: all tissues



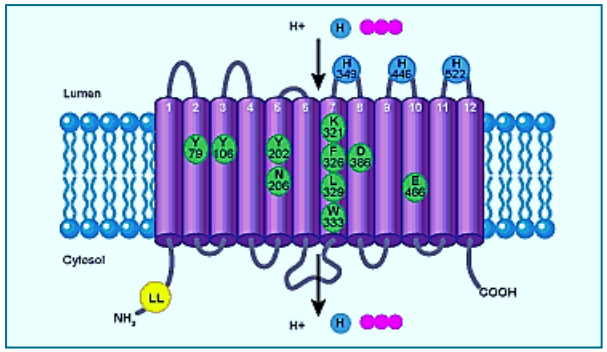
- ABCG2: placenta, liver, intestine.

ABC transporters - Role

- Physiological role : Lipid transport and endogenous compounds
  - Cholesterol, phospholipids, interleukins, …
- Multidrug resistance – Cancer drug resistance:
  - Overexpression of MDR1, MRP1 and BCRP
- Protection against xenobiotics,
  - Limit the absorption of many drugs from the intestine
  - Pump drugs from the liver and kidney cells as a means of removing foreign substances from the body
  - Passage of drugs through cellular and tissue barrier
- Drug disposition
  - Significantly modulate the absorption, distribution and elimination
  - Efficacy and toxicity of pharmacological agents
- Drug-drug interactions (DDI)

SLC transporter Superfamily

- 386 SLC human genes
  - grouped into 52 families



Mutagenetix Labarchives

UPTAKE

- SLC transporters include families of :
  - SLCO: Organic anion transporting polypeptide
  - SLC22: Organic anion/cation/zwitterion transporter

Drug uptake into intestine, liver, and kidney,

  - SLC47: Multidrug and toxin extrusion (MATE)

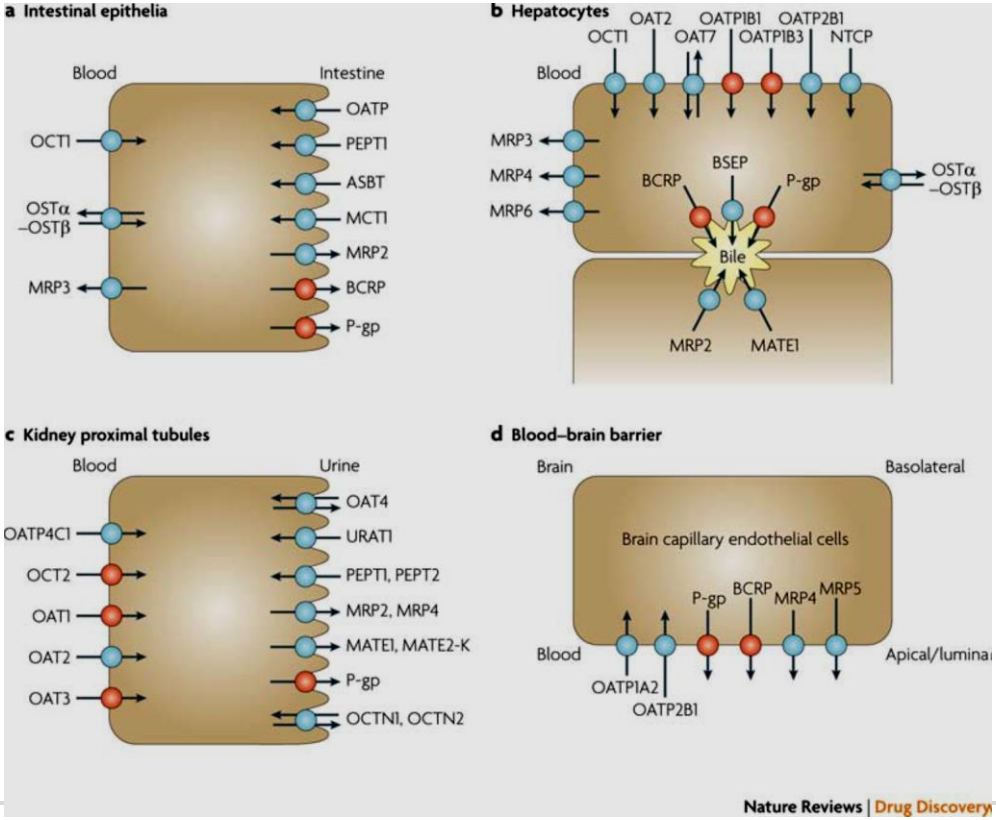
Drug efflux into bile and urine

SLC transporters

- Tissue Distribution:
  - Highly abundant in the intestine, liver, kidney,
- Physiological role:
  - Transport of steroid conjugates, thyroid hormones, bile saltes,
- Regulate drug Pharmacokinetics:
  - absorption, distribution, and excretion of drugs
- Mediate drug-drug interactions
- Genetic variation in SLC genes showed to contribute to interindividual pharmacokinetic and pharmacodynamic variability
  - Example of Statins (Pravastatin / SLCO1B1)

Co-localization of SLC and ABC transporters

- Co-localization of ABC and SLC transporters (and CYP enzymes) in many key tissues
  - Very complex orchestra for body protection that impact drug disposition



ITC Whitepaper, 9:215-236, 2010

Regulatory perspectives

- Due to contribution of drug transporters to drug-drug interactions, European and US regulatory agencies require evaluation of drug transporters for the substrate and inhibition potential of drug candidates.
- FDA (Guidance 2012):
  - 7 key transporters: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2
- EMA (Guidance 2013)
  - 9 key transporters: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2 and OCT1 (and BSEP)
- Very recently, evaluation of MATE transporters is required by the regulatory agencies.

Regulatory perspectives

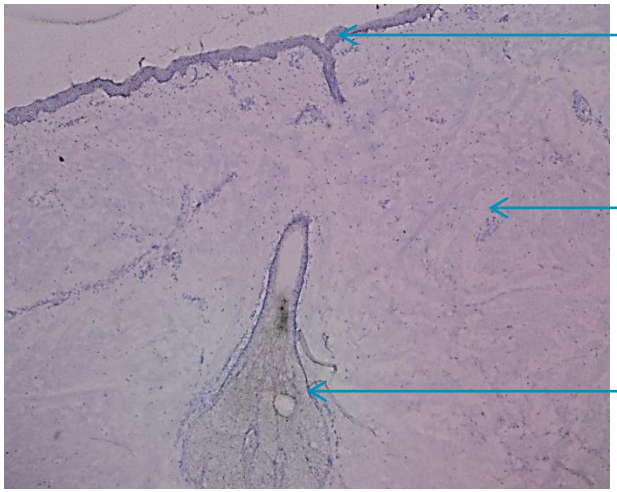
- ABC and SLC transporters are well characterized in liver, kidney and intestine but little is known about skin.

- Objective of this work was the characterization of drug transporters in human skin :
  - Expression
  - Regulation
  - Localization
  - Role in drug disposition

- In order to meet regulatory agencies requirement for topically applied drugs.

Drug transporters in Human skin

- Skin is the largest organ of the body:
  - 2 m^2 surface area,
  - 0.5 – 4 mm thickness
  - 16% body weight



- Skin plays a crucial role in body protection from:
  - damage, infection and drying out.

Human skin

- Skin is not only a physical barrier but also a biochemical barrier

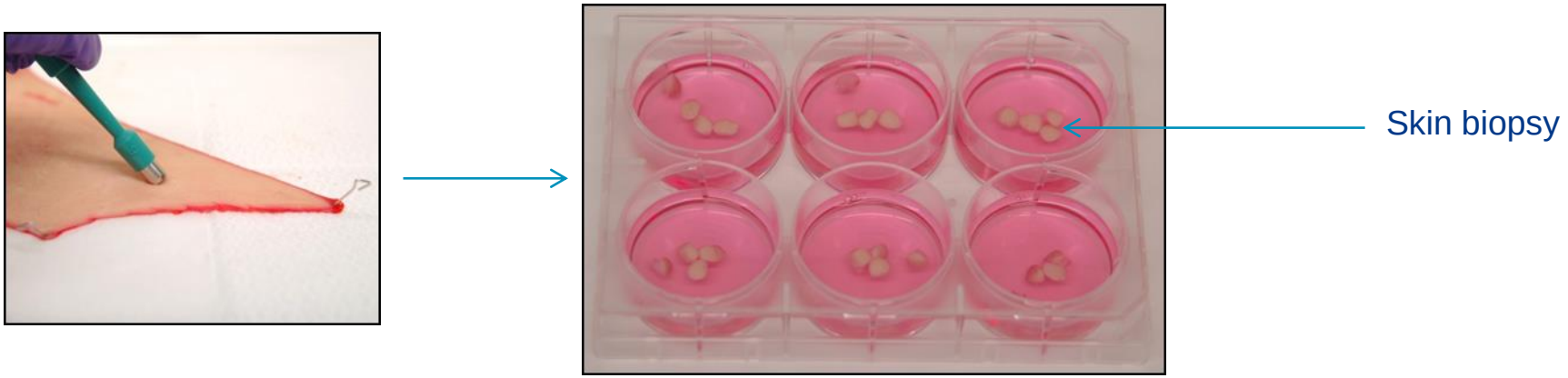
- Expression of drug metabolizing enzymes in the skin is well documented

2nd Skin Metabolism Meeting
Valbonne, France, 10 - 11 October 2013

- Expression and role of drug transporters in human skin are poorly understood.

Expression of ABC and SLC transporters in human skin

- Human skin biopsies in organ-culture for 3 days



- 4 skin biopsies (6 mm diameter) per well of 6-well plates.
- Culture medium: Long term skin culture medium (Biopredic, France).
- RNA extraction followed by quantitative real time RT-PCR (TaqMan technology).
- GAPDH gene used as housekeeping gene

Expression of ABC and SLC transporters in human skin

- 11 SLC and 4 ABC transporters genes were evaluated
- Expression levels measured in human skin, and compared with human liver and kidney.

SLC transporter genes

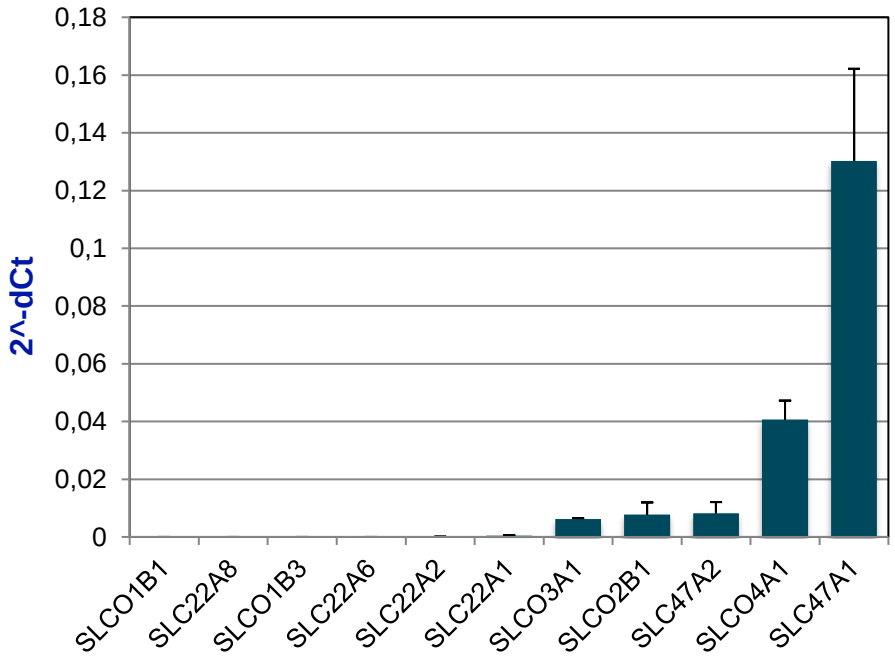
Gene	Transporter
SLCO1B1	OATP1B1
SLCO1B3	OATP1B3
SLCO2B1	OATPB
SLCO3A1	OATPD
SLCO4A1	OATPE
SLC22A1	OCT1
SLC22A2	OCT2
SLC22A6	OAT1
SLC22A8	OAT3
SLC47A1	MATE1
SLC47A2	MATE2

ABC transporter genes

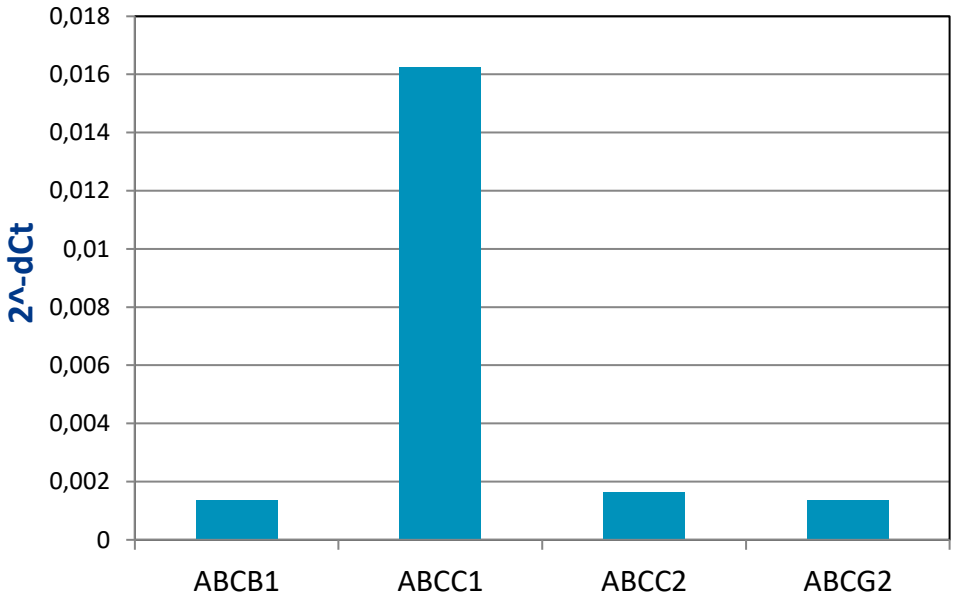
Gene	Transporter
ABCB1	MDR1
ABCB1	MRP1
ABCC2	MRP2
ABCG2	BCRP

Expression of ABC and SLC transporters in human skin

SLC transporters
in human skin
(N = 3 donors)



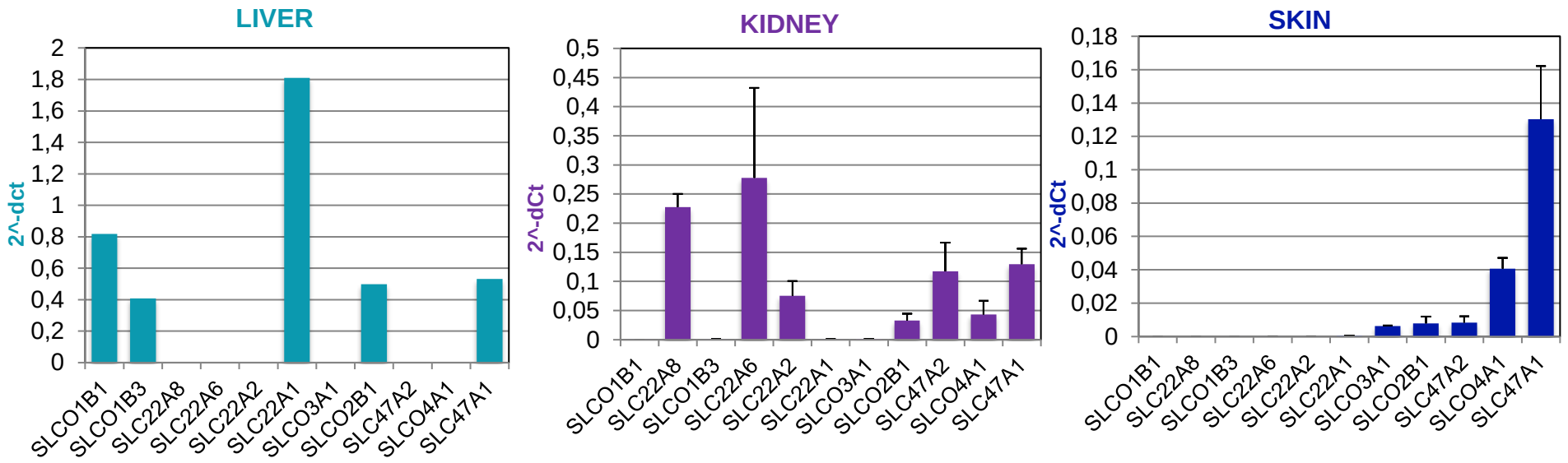
ABC transporters
in human skin
(N = 8 donors)



- 5 SLC transporters over 11 are expressed in human skin, MATE1 (SLC471) is the most abundant.
- All the 4 ABC transporters are expressed in human skin with MRP1 (ABCC1) is the most expressed.

Expression of ABC and SLC transporters in human skin

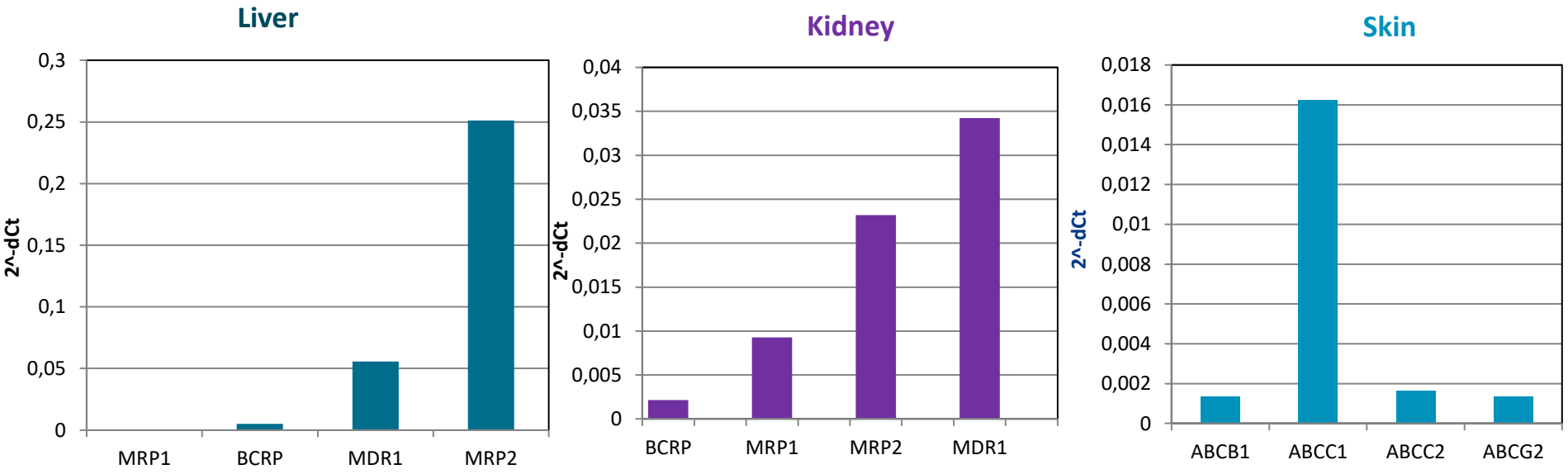
Comparison of SLC transporters in Skin, Liver and kidney



- Expression profile very different according to tissue
  - SLC47A1 (MATE1) most expressed in skin
  - SLC22A1 (OCT1) most expressed in liver
  - SLC22A6 (OAT1) most expressed in kidney

Expression of ABC and SLC transporters in human skin

Comparison of ABC transporters in Skin, Liver and kidney

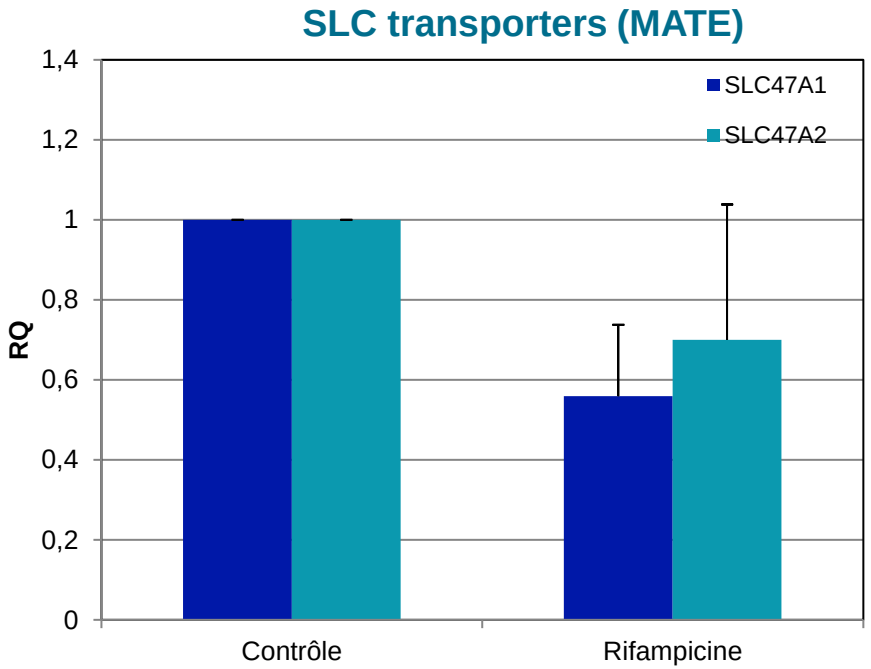


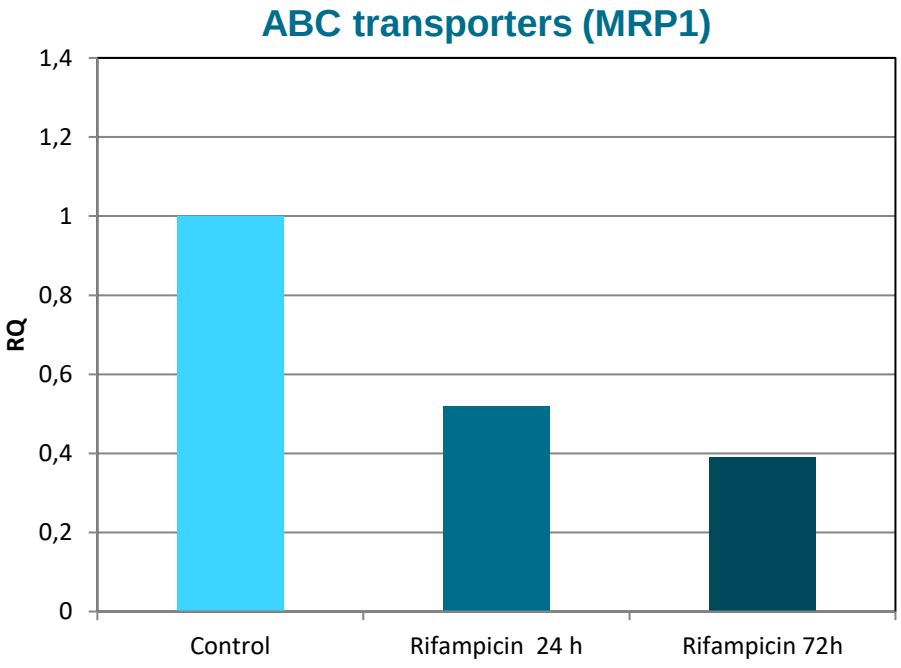
- Expression profile of ABC transporters very different according to tissue
  - ABCC1 (MRP1) most expressed in skin
  - ABCC2 (MRP2) most expressed in liver
  - ABCB1 (MDR1) most expressed in kidney

Regulation of ABC and SLC transporters in human skin

Effect of Rifampicin on ABC and SLC transporters in Skin

Rifampicin: 50 µM during 72 h
Human skin biopsies in organ-culture
N = 2 or 3 donors



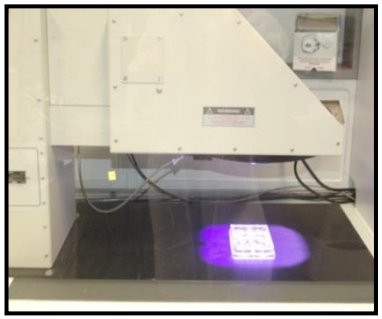


- Rifampicin markedly decreases expression of MATE and MRP1 transporters in human skin.

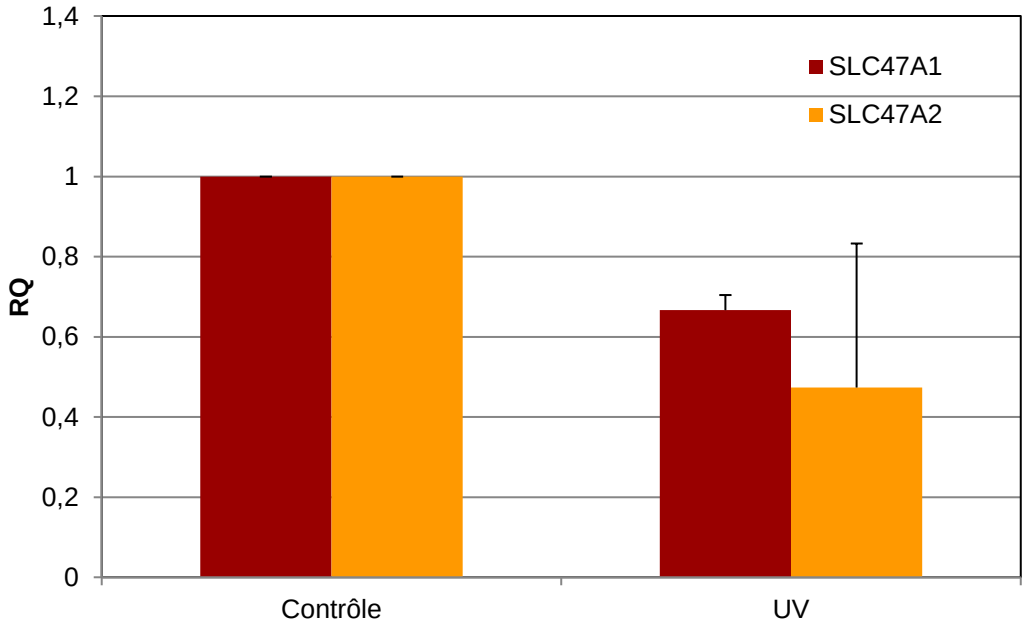
Regulation of ABC and SLC transporters in human skin

Effect of UV irradiation on MATE transporters

Human skin biopsies in organoculture
N = 2 donors
Solar simulator
Irradiation during 1 hour per day for 3 days.



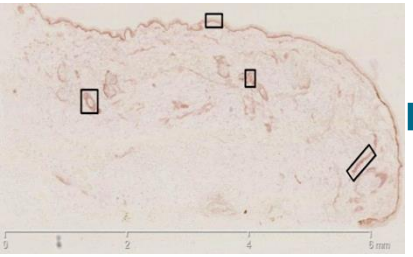
Solar simulator
(830W ; 47.8A;
UVA 110W/m²
UVB: 20W/m²)



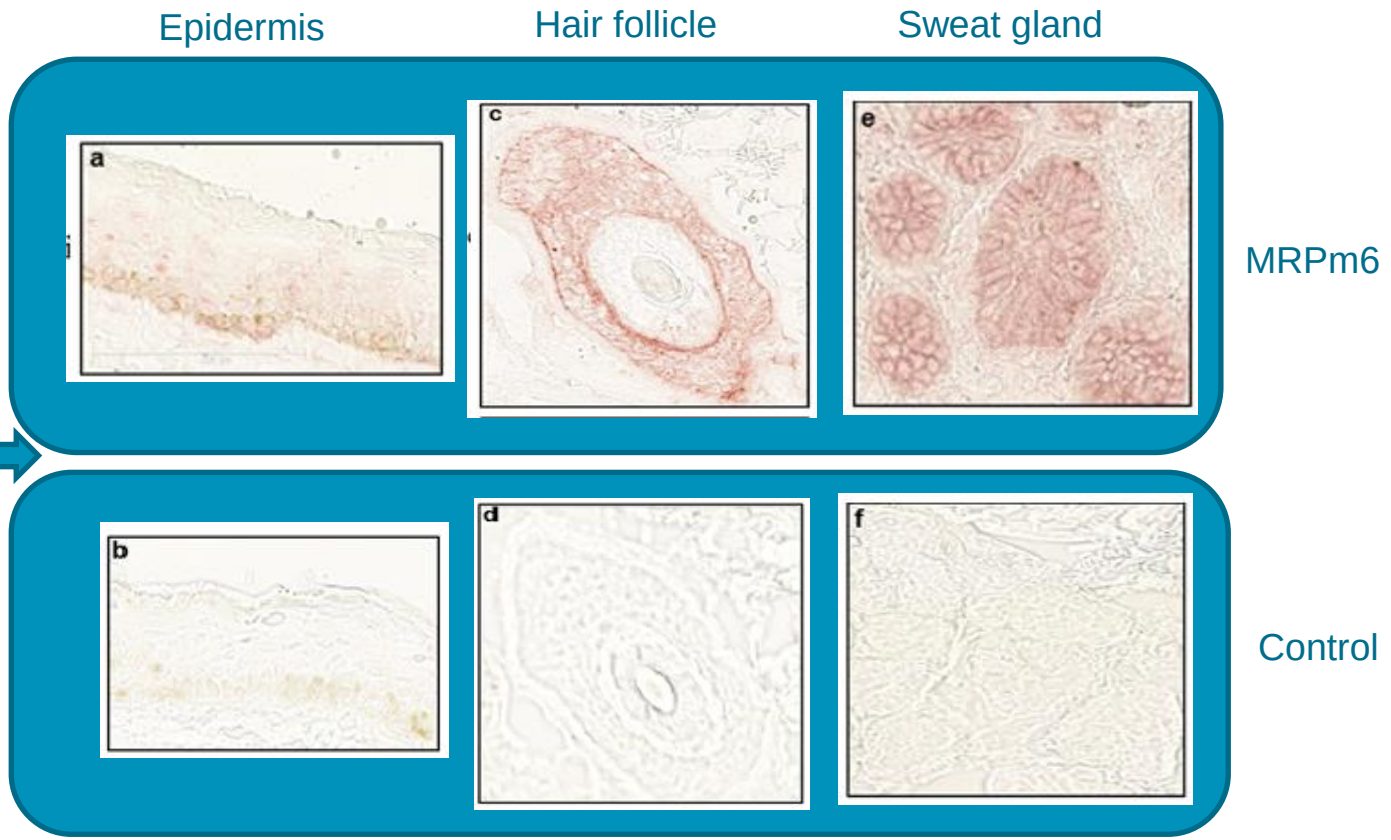
- UV irradiation markedly decreases expression of MATE1 and MATE2 transporters in human skin.

Localization of MRP1 in human skin

- Immunohistochemical analysis of MRP1 in human skin
- Cryosection of skin, and MRPm6 monoclonal antibody.



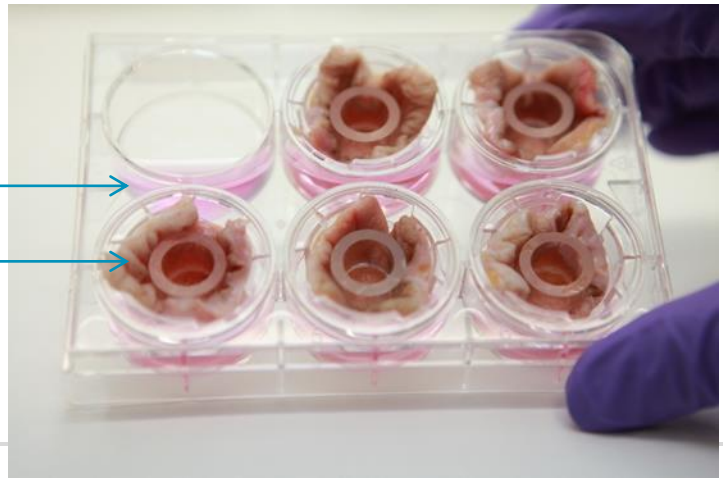
Human skin cryosection



- MRP1 is mainly localized in the hair follicle and sweat gland in the dermis

Role of MRP1 transporter in drug absorption in the skin

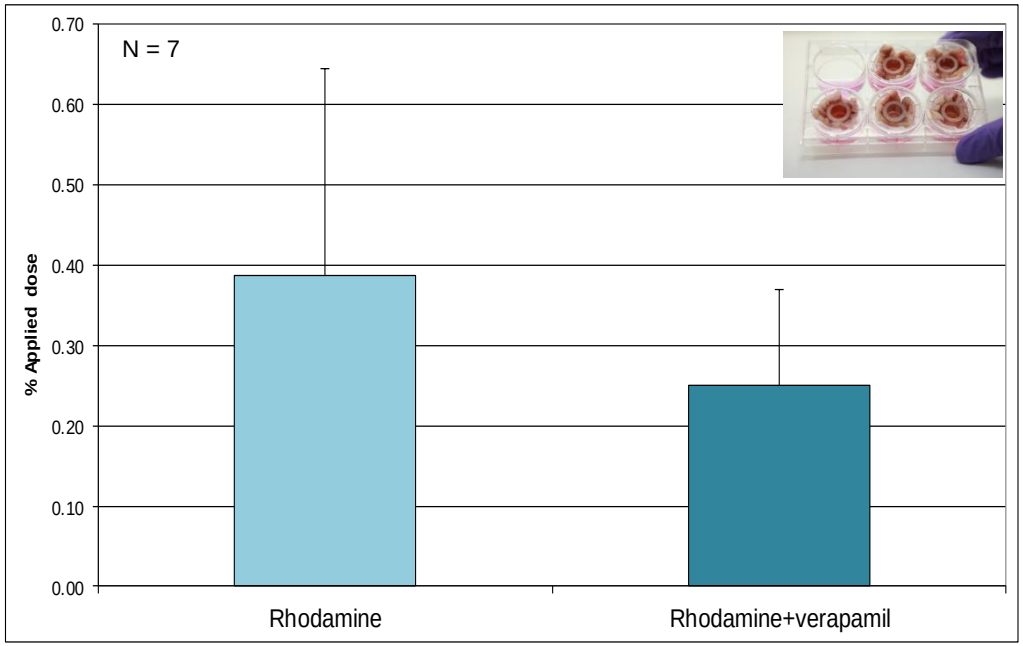
- Skin absorption and distribution of 3 couples of MRP1 substrates and inhibitors were evaluated:
  - Rhodamine 123 / Verapamil
  - [^{3}H]-Vinblastine / Verapamil
  - [^{3}H]-LTC4 / MK571
- *In vitro* model for skin absorption
  - Donor compartment (skin) on Transwell porous membrane
  - Receptor compartment (culture medium)



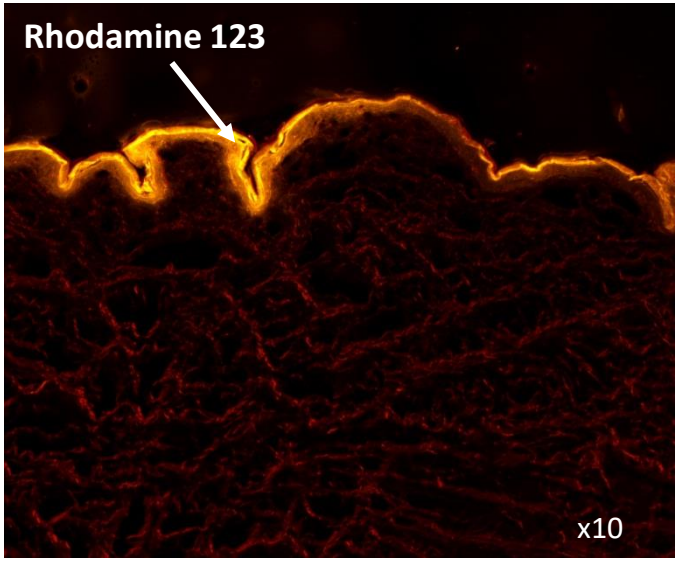
In vitro skin absorption model

Skin absorption of Rhodamine 123

Skin absorption



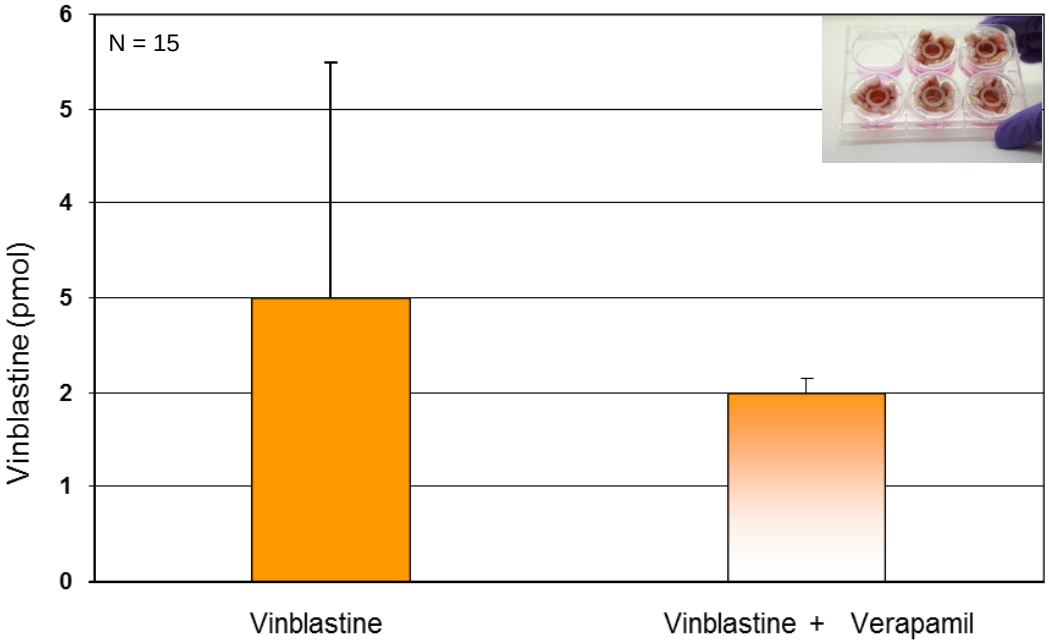
Skin distribution
(Epifluorescent microscopy)



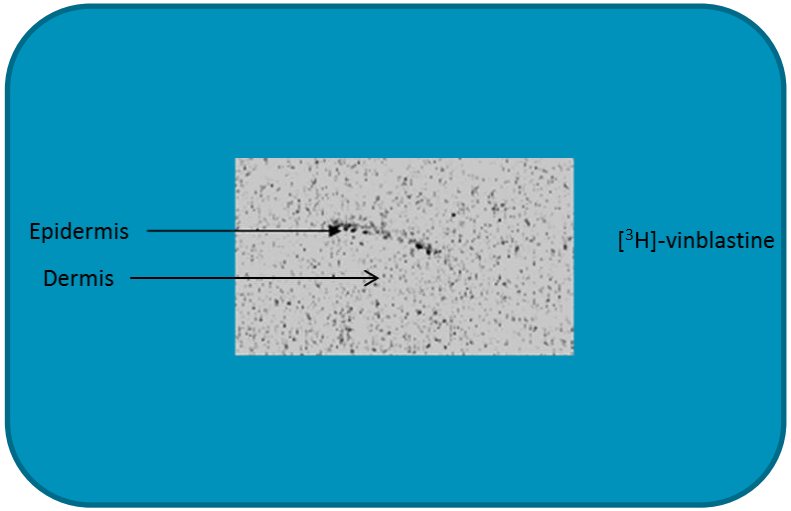
- Inhibition of MRP1 by verapamil significantly decreases skin absorption of Rhodamine 123.
- Rhodamine 123 mainly distributed in the epidermis.

Skin absorption of Vinblastine

Skin absorption



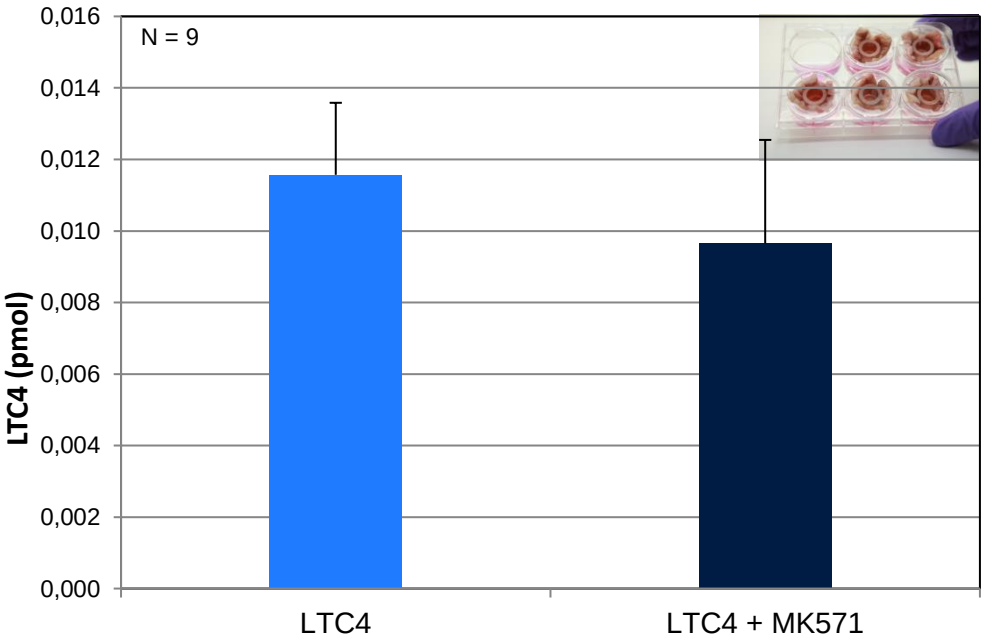
Skin distribution (Autoradiography)



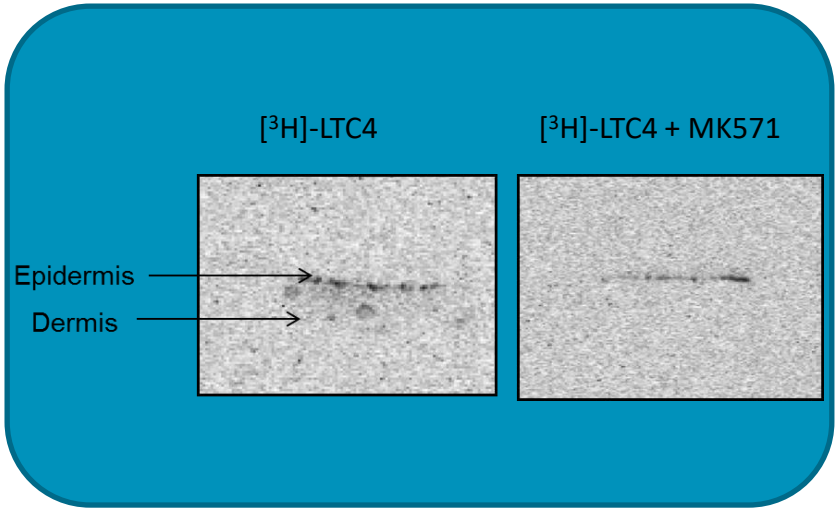
- Inhibition of MRP1 by verapamil significantly decreases skin absorption of vinblastine.
- Vinblastine mainly distributed in the epidermis

Skin absorption of LTC4

Skin absorption



Skin distribution (Autoradiography)



- Inhibition of MRP1 by MK571 significantly decreases skin absorption of LTC4.
- Vinblastine distributed in the epidermis and the dermis.

Conclusions

- Expression and regulation of drug transporters were shown in human skin
  - Expression profile different in skin, liver and kidney
- MRP1 is mainly localized in the dermis (hair follicle, sweat gland), and play a key role in drug uptake in human skin
- Further studies needed to clarify the role of drug transporters in clinical drug-drug interactions with topically applied drugs.

H. Osman-Ponchet et al., 2014, Drug Metabolism and drug interactions

M. Alriquet et al., 2015, ADMET & DMPK

Thank you

Thank to my team

- Anaïs Boulai
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