Pig skin as an alternative to human skin for skin metabolism studies?

H. Osman-Ponchet, A. Lemoine, A. Gaborit, K. Sevin, M. Alriquet, P. Comby, B. Ruty

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

hanan.osman-ponchet@galderma.com

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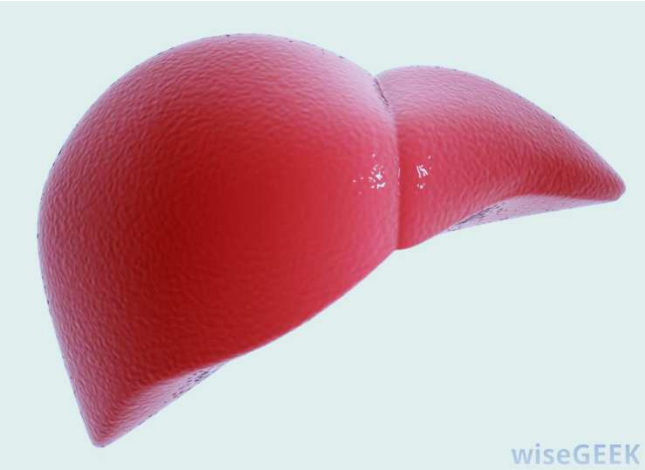
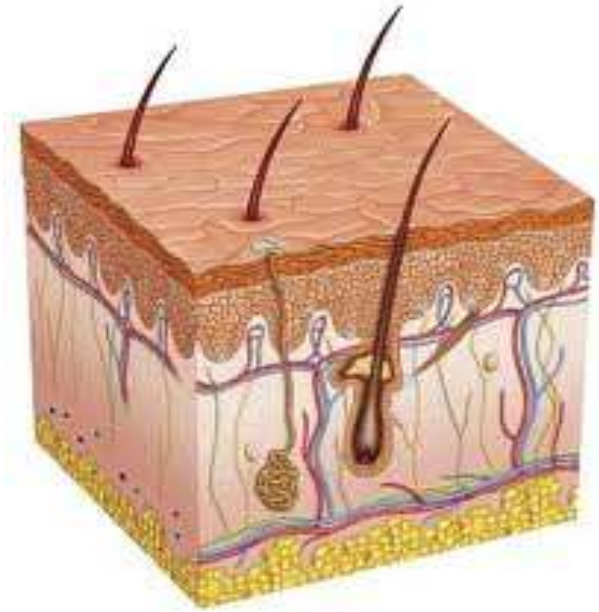
Outline of the presentation

- General overview of drug Metabolism
  - Importance of skin metabolism
  - Localization of drug metabolizing enzymes in the skin
  - How to evaluate skin metabolism? Which model?
  - Comparison skin and liver metabolism?
- Characterization of drug metabolizing enzymes in:
  - Human skin and human liver
  - Minipig skin and minipig liver
  - Comparison of Testosterone metabolism in Human and Minipig
- Conclusion

Importance of skin metabolism

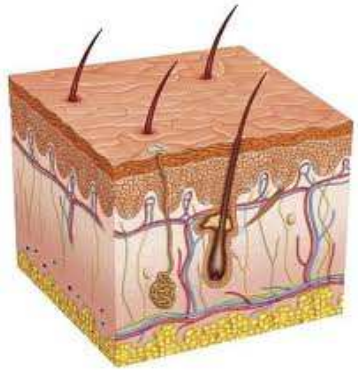
- Drug metabolism mainly occurs in the liver





- Drug metabolism may occur in the skin (2nd Skin Metabolism Meeting)

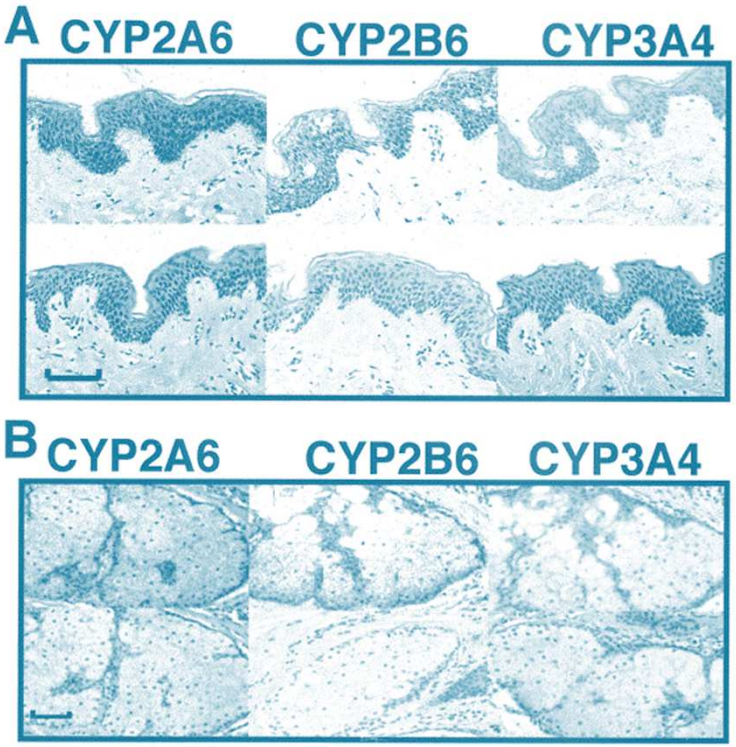
Importance of skin metabolism



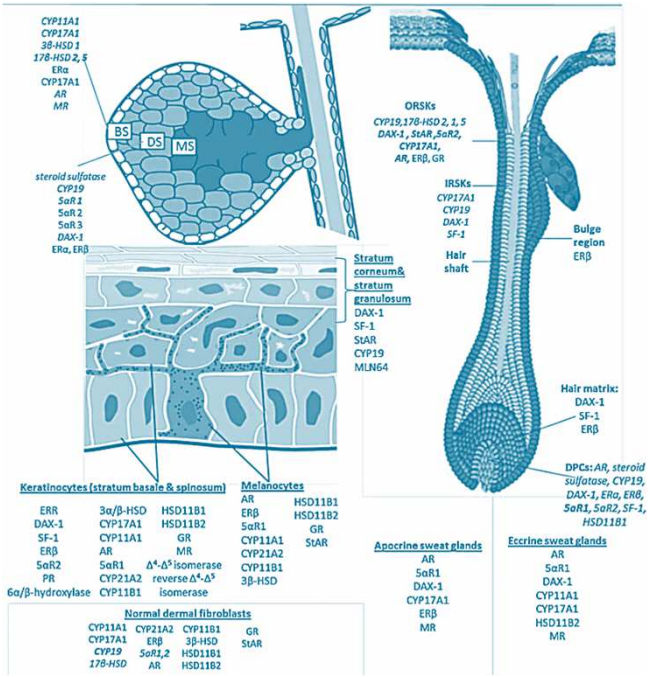
- Skin is a biochemical barrier:
  - Expression of metabolizing enzymes
    - Phase I (CYPs, FMOs, esterases, ADH…)
    - Phase II (UGT, GST, NAT…)
  - Lower expression in the skin compared to the liver
  - May be induced as in the liver
  - Expression of drug transporters
    - ABC transporters : MRP1, MDR1 (Osman-Ponchet et al., 2013, Drug Metabolism and Drug Ineractions)
    - SLC transporters : MATE (Alriquet et al., DDI 2013, Marbach Castle, Germany)
- Influence on drug delivery
  - Ester & other prodrugs (enhancement of absorption by ester hydrolysis)
  - Drug-drug interactions (limited available data!)
- Influence on drug toxicity
  - Skin sensitization
  - Toxic metabolites

Localisation of drug metabolizing enzymes in the skin

- Epidermis
- Dermis, Hair follicles, sebaceous gland,



Janmohamed et al., 2001 Biochemical Pharmacology



Slominski et al. 2013,
The journal of steroid biochemistry and molecular biology

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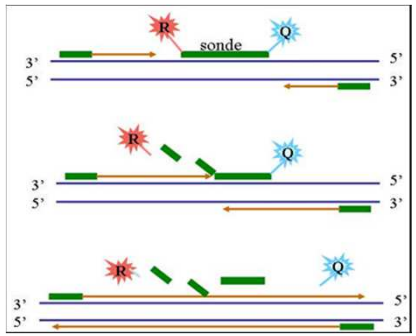
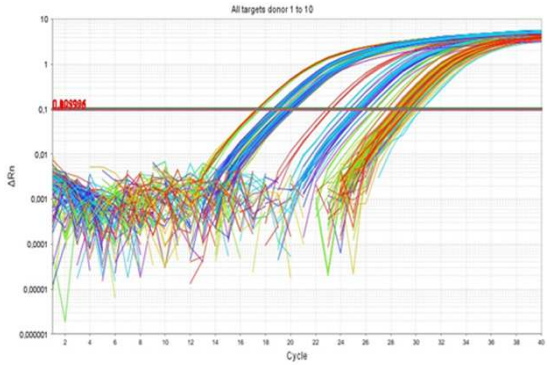
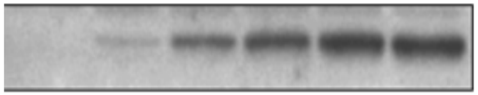
How to evaluate skin metabolism?

- Approaches used to study skin metabolism:
  - Gene expression (mRNA)

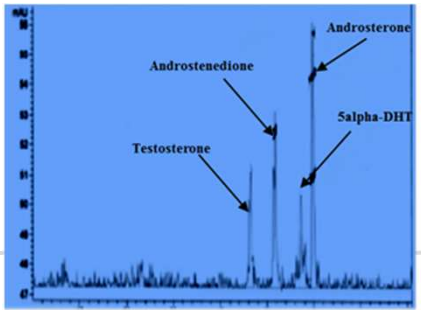




  - Protein expression



  - Enzyme activity using specific substrates and inhibitors
    - (Km, Vmax)
  - Functional studies to give metabolites



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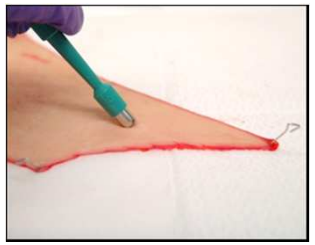
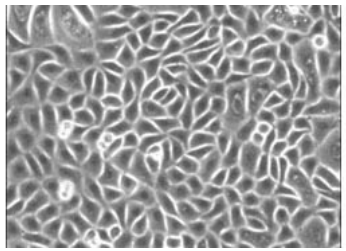
Skin metabolism : Which model?

- Models used to study skin metabolism:
  - Whole skin
    - Freshly excised or in organoculture



  - Reconstructed Human skin

  - Isolated cells



  - Skin Homogenates

  - Skin Microdialysis

Comparison skin and liver

- Few data comparing skin and liver metabolizing enzymes in Human
  - Liver >> skin
- For a given enzyme, it is very important to know the relative concentration in the skin and the liver for the same subject
  - Are all these enzymes present at the same level in each organ?
  - Does the sex or the animal species influence the expression of these enzymes?

Objectives

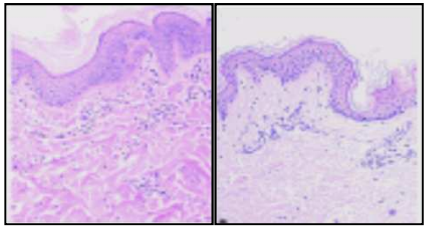
1. To compare the expression of the main drug metabolizing enzymes in skin and liver in human (same subjects)

2. To compare the expression pattern in human and porcine skin and liver

➡ Minipig:

- Nonrodent species in regulatory toxicology
  - Bodes et al., 2010, J. Pharmacoll and Toxicological Methods

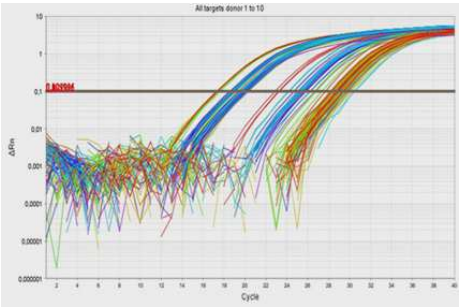
- Similarities/differences between porcine and human skin
  - Age-dependent skin thickness
  - Lower elastic fibers
  - Apocrine sweat glands…
  - Liu et al., 2010, Comparative Medicine



Porcine skin Human skin

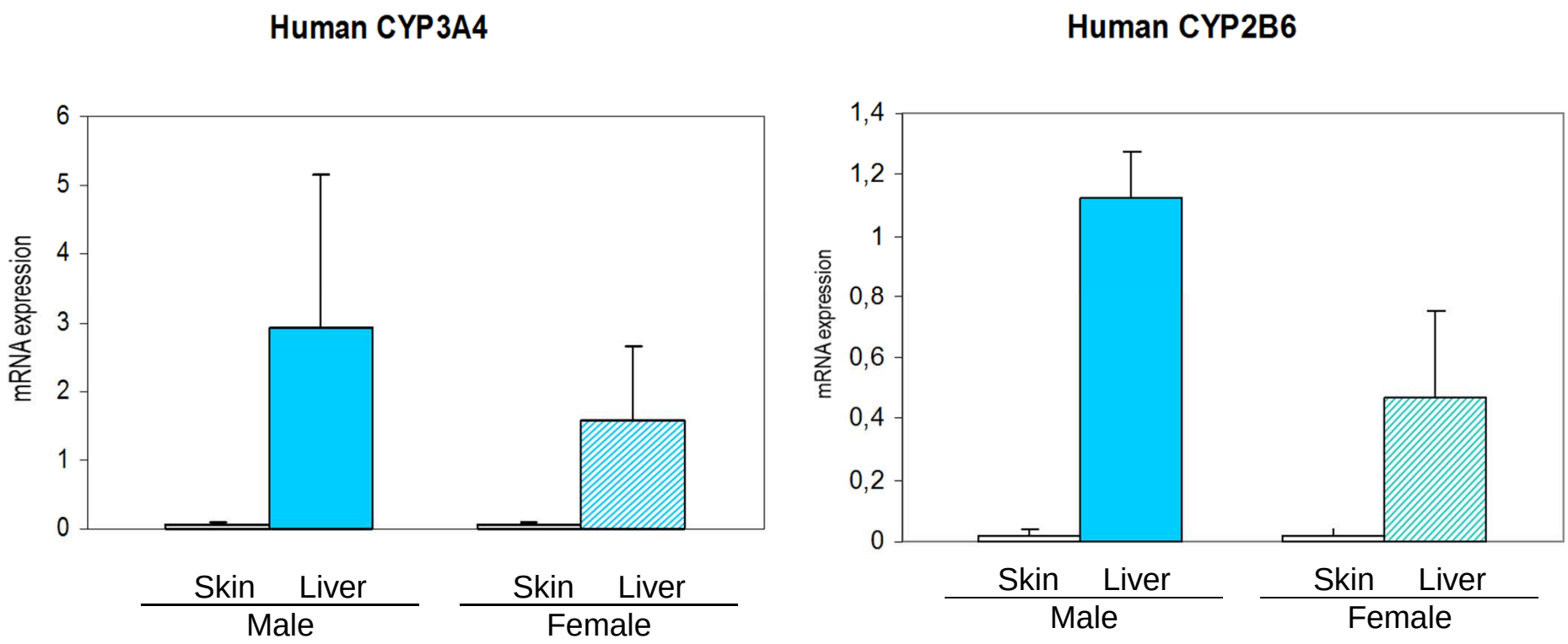
Experimental procedure

- Six couples of human skin and liver biopsies
  - 3 women and 3 men
- Surgery of hepatic metastatses (colon cancer)
- Immediately frozen in liquid nitrogen
- Crushing of biopsies in liquid nitrogen
- Total RNA extraction (kit RNeasy Quiagen)
  - 80 to 1000 ng ARN / mg tissue,
  - very good quality,
  - variable according to sample with liver > skin
- 8 couples of minipig skin and liver
  - 4 females and 4 males (Göttingen minipig)
- Real-time quantitative RT-PCR
- CYP1A1, CYP3A4, CYP2B6, CYP2D6, hFMO2, hFMO3, hFMO5
  - Beta-actin: reference gene



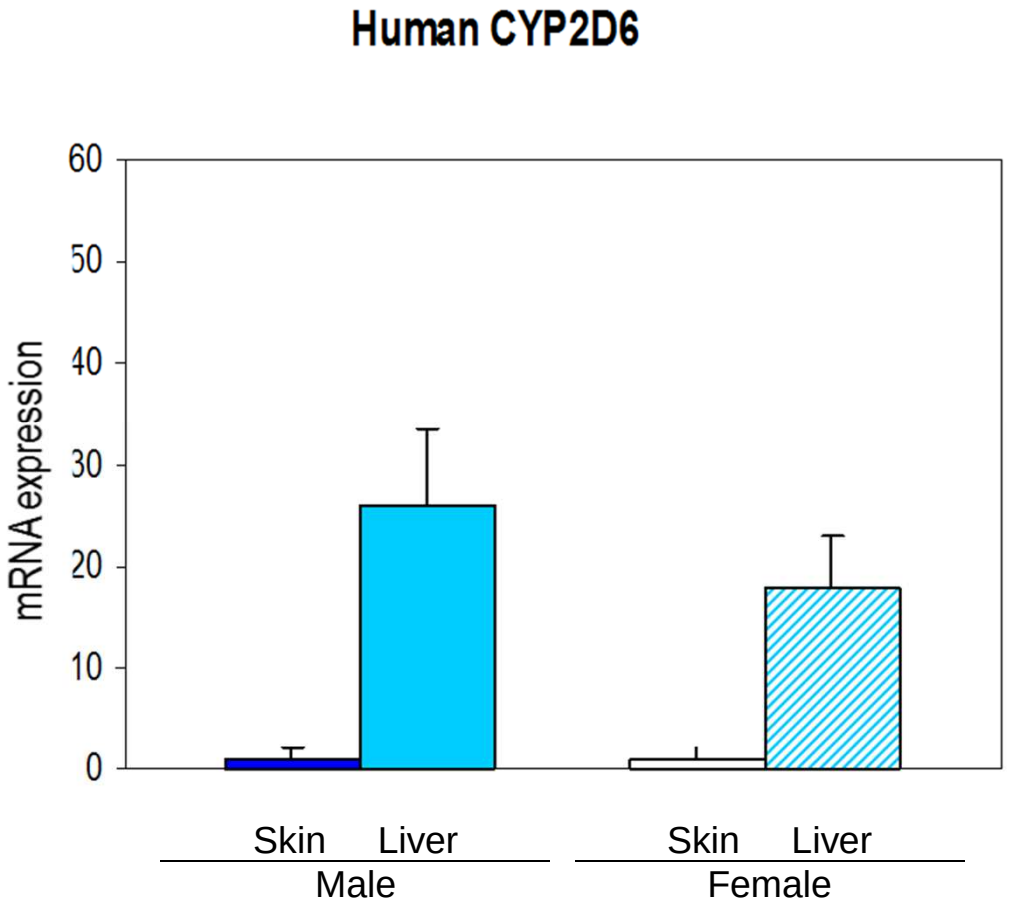
RESULTS

Expression level of CYP in Human skin and liver



Very low mRNA expression level of CYP3A4 and CYP2B6 in human skin compared to liver

Expression level of CYP in Human skin and liver



Very low mRNA expression level of CYP2D6 in human skin compared to liver

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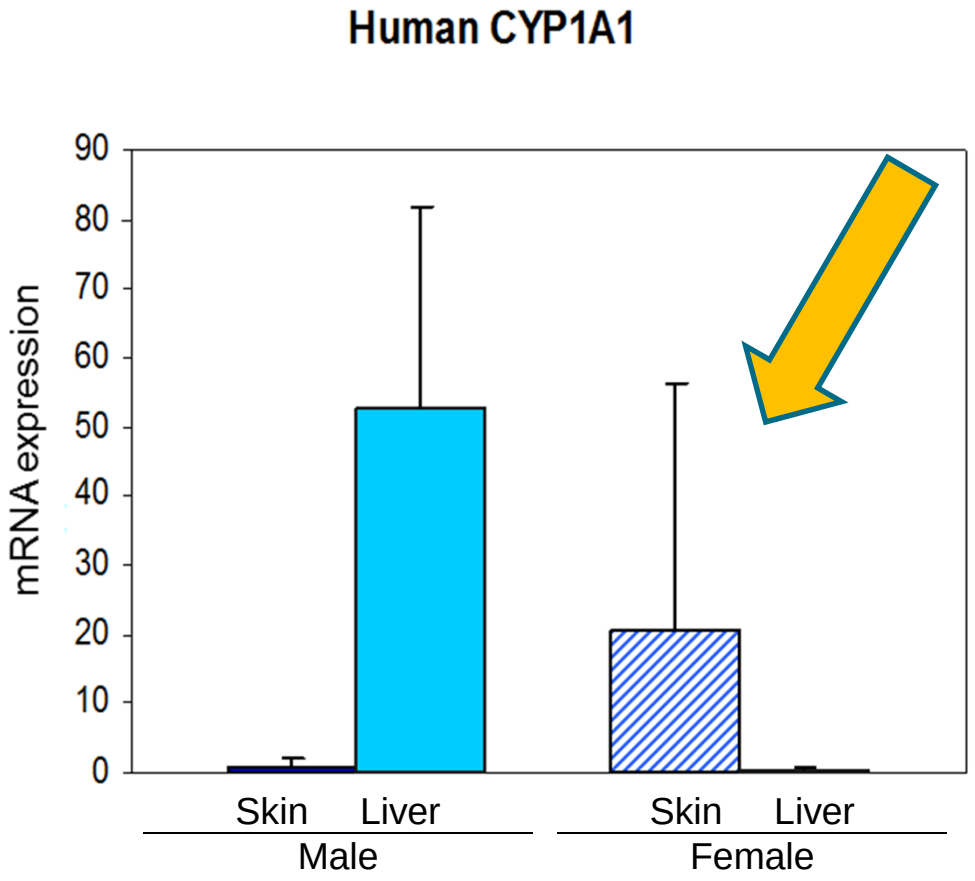
Expression level of CYP in Human skin and liver



Gender effect in Liver and skin

- CYP1A1: Liver >> Skin (Male)
- CYP1A1: Skin >> Liver (Female)

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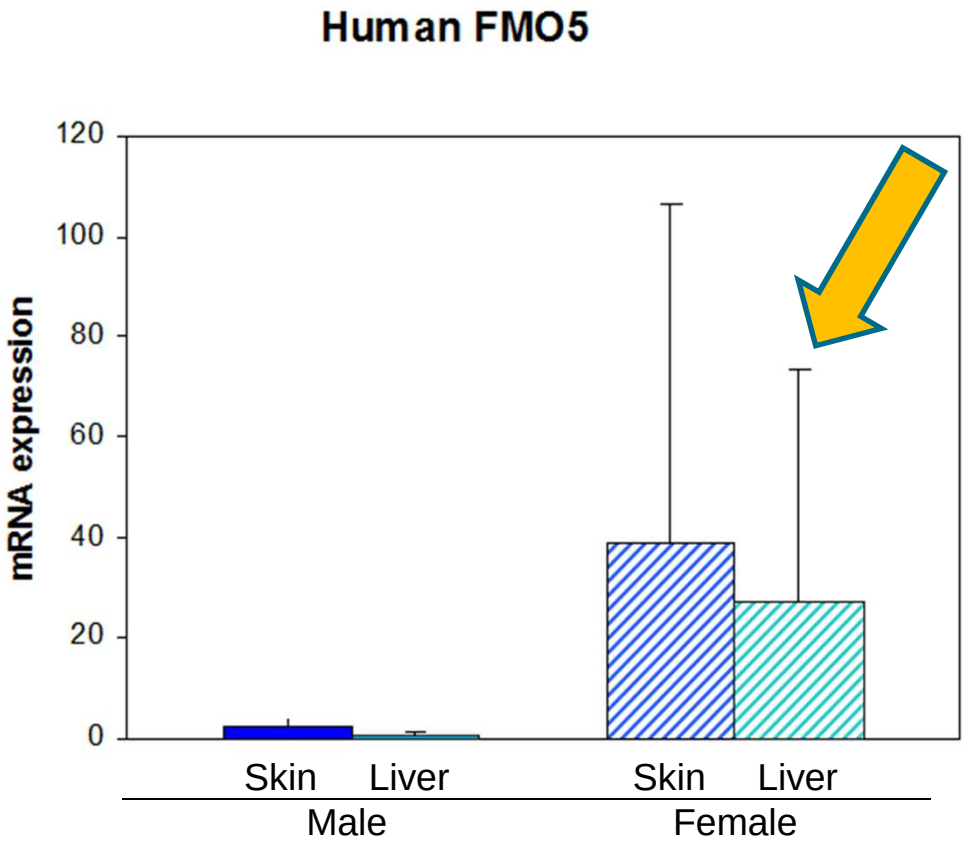
Expression level of FMO5 in Human skin and liver

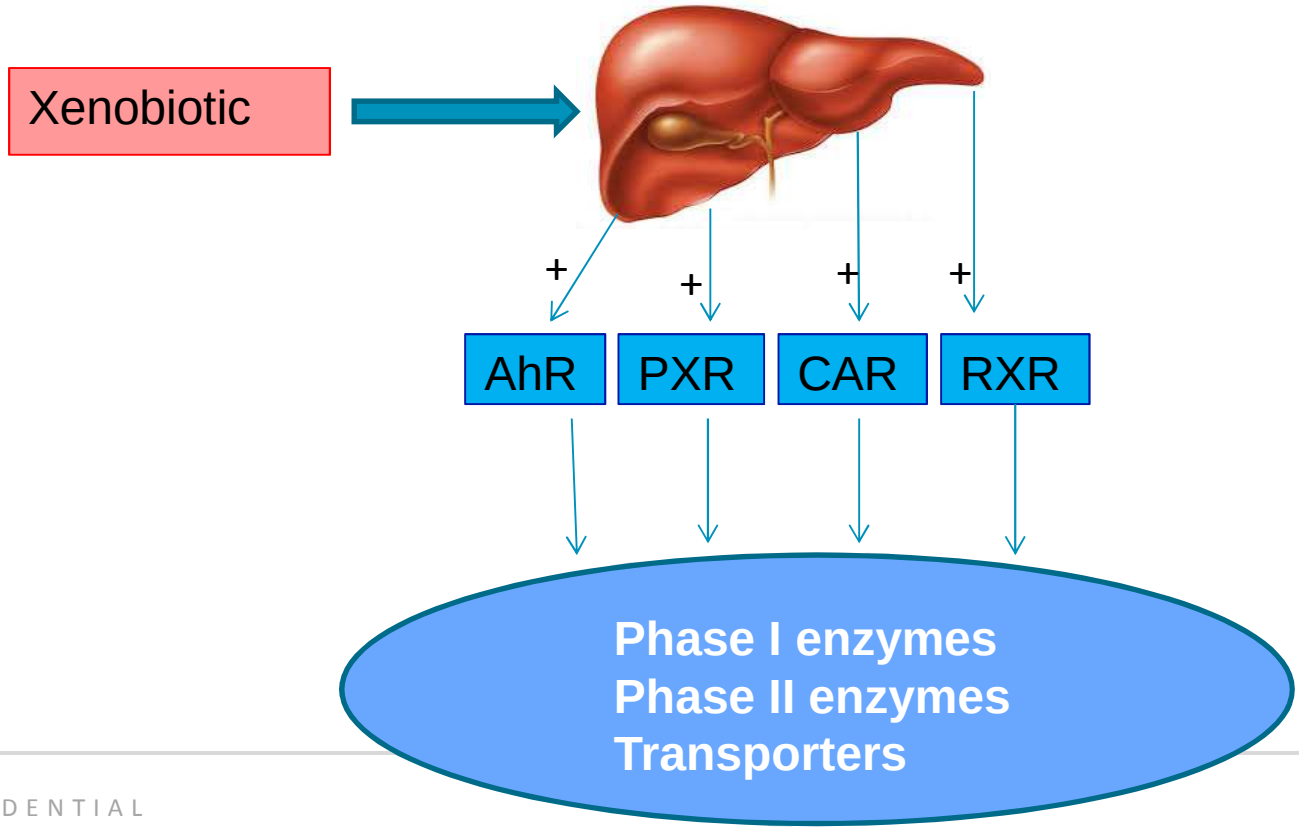


High variability:
Small size of sample

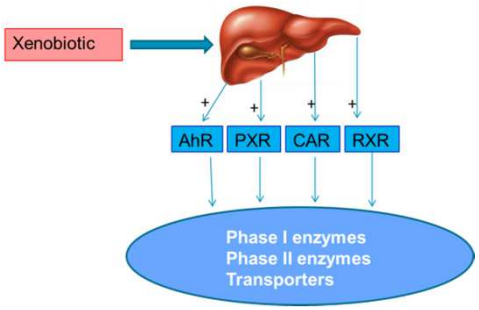
FMO5: Female >> Male
FMO5: Skin ≥ Liver

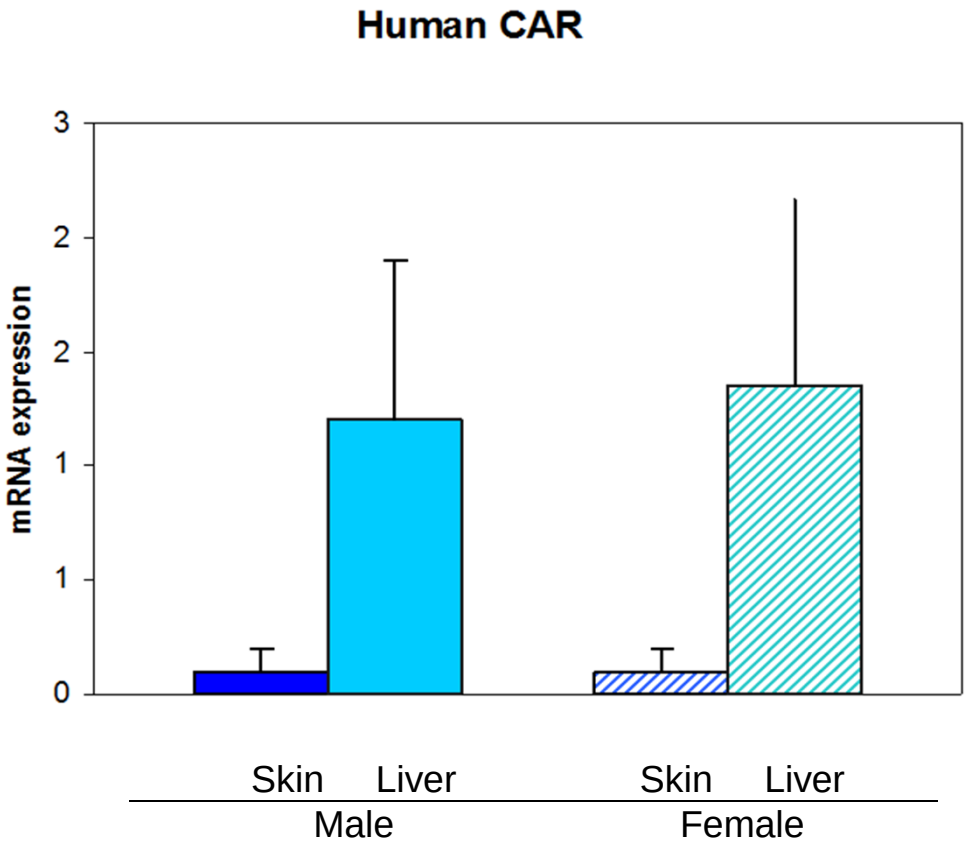
Regulation of drug metabolizing enzymes by nuclear receptors

- Metabolizing enzymes genes are induced through a ligand-activated nuclear receptor:
  - Pregnane X receptor (PXR)
  - Aryl hydrocarbon receptor (AhR)
  - Constitutive androstane receptor (CAR)



Expression level of CAR in Human skin and liver





Very low mRNA expression level of CAR in human skin compared to liver

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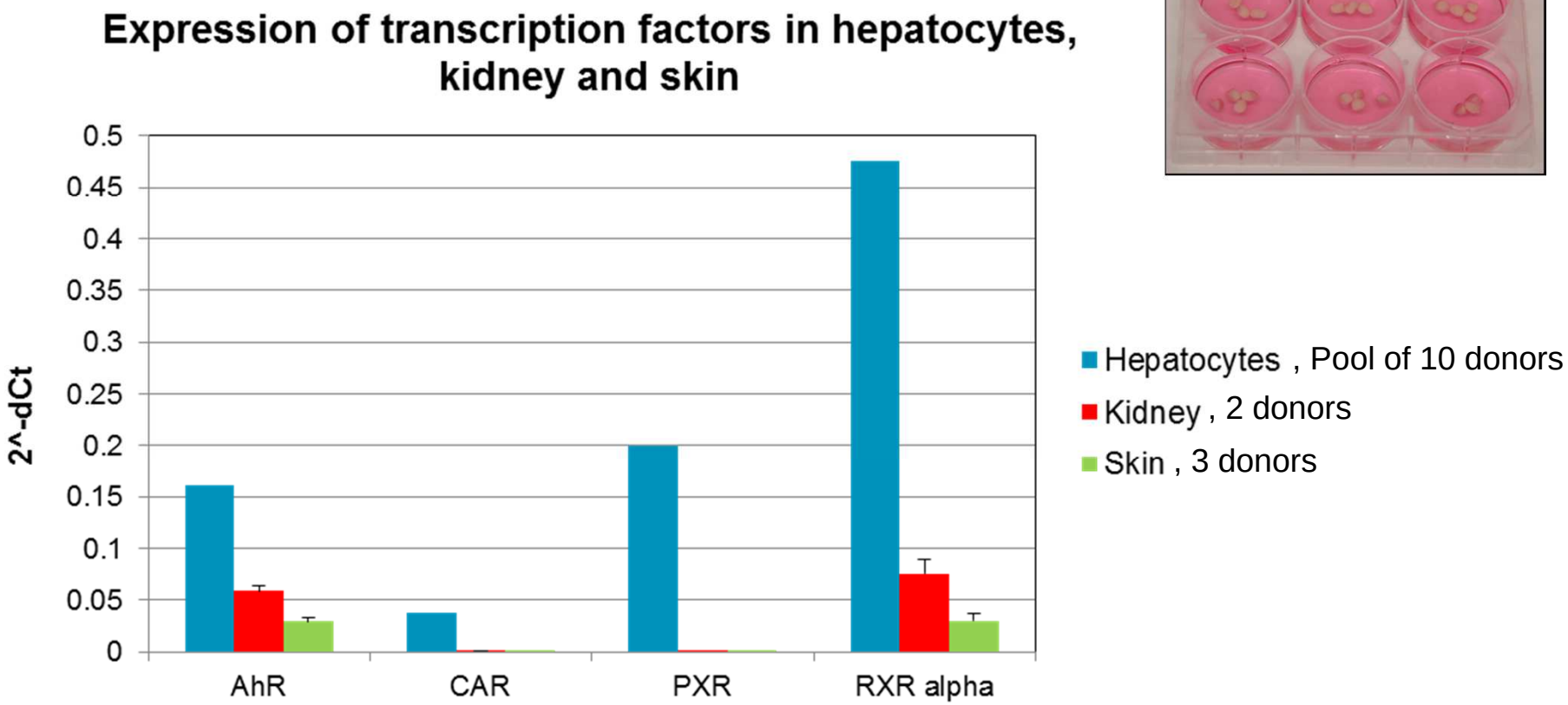
Expression level of nuclear receptors in Human skin, kidney and liver



Expression level of CAR and PXR is very low in human skin maintained in organoculture compared to AhR and RXR alpha

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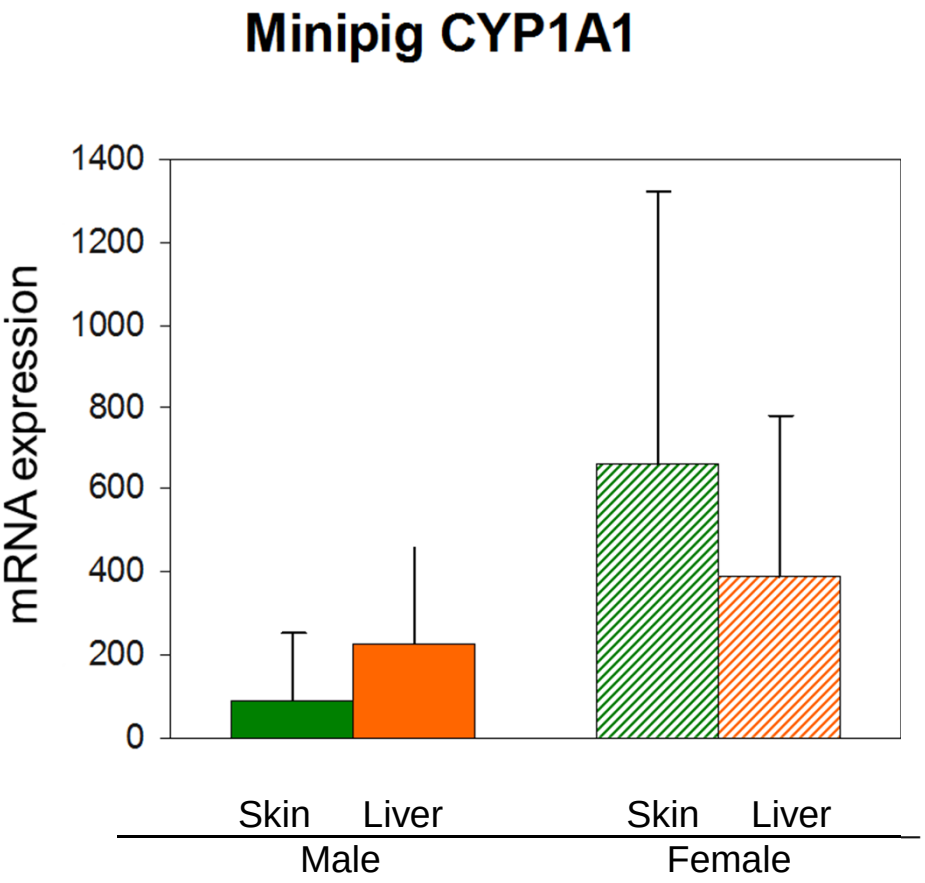
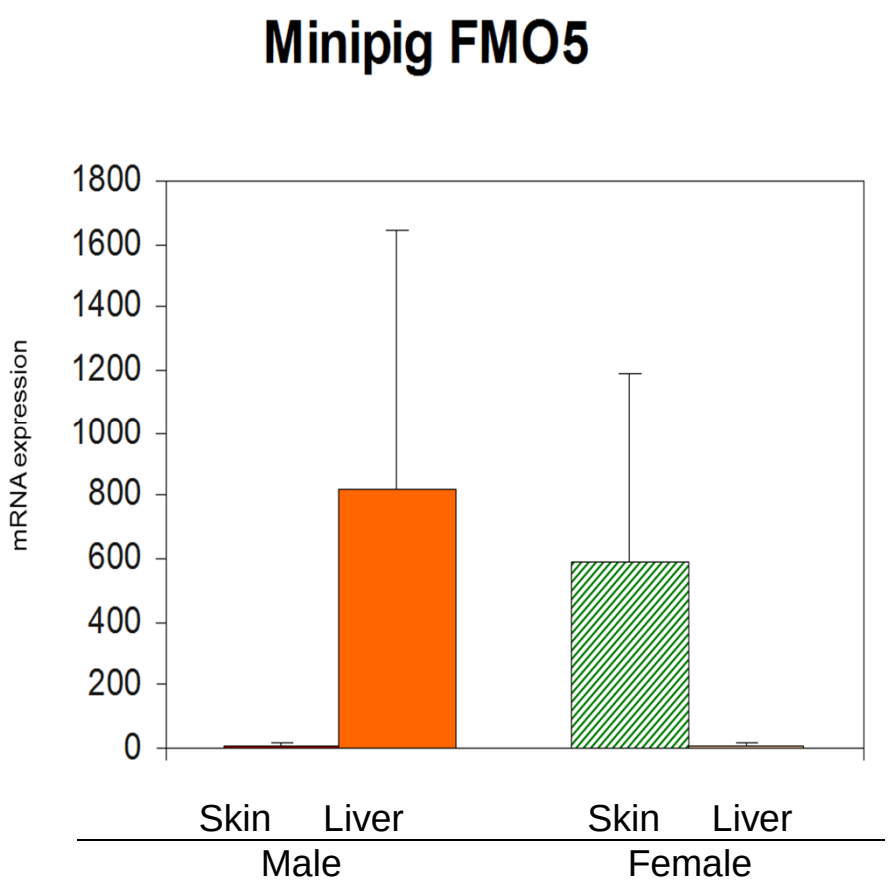
Expression level of drug metabolizing enzymes in Minipig





CYP1A1: Female > Male
FMO5: Skin >> Liver (Female)

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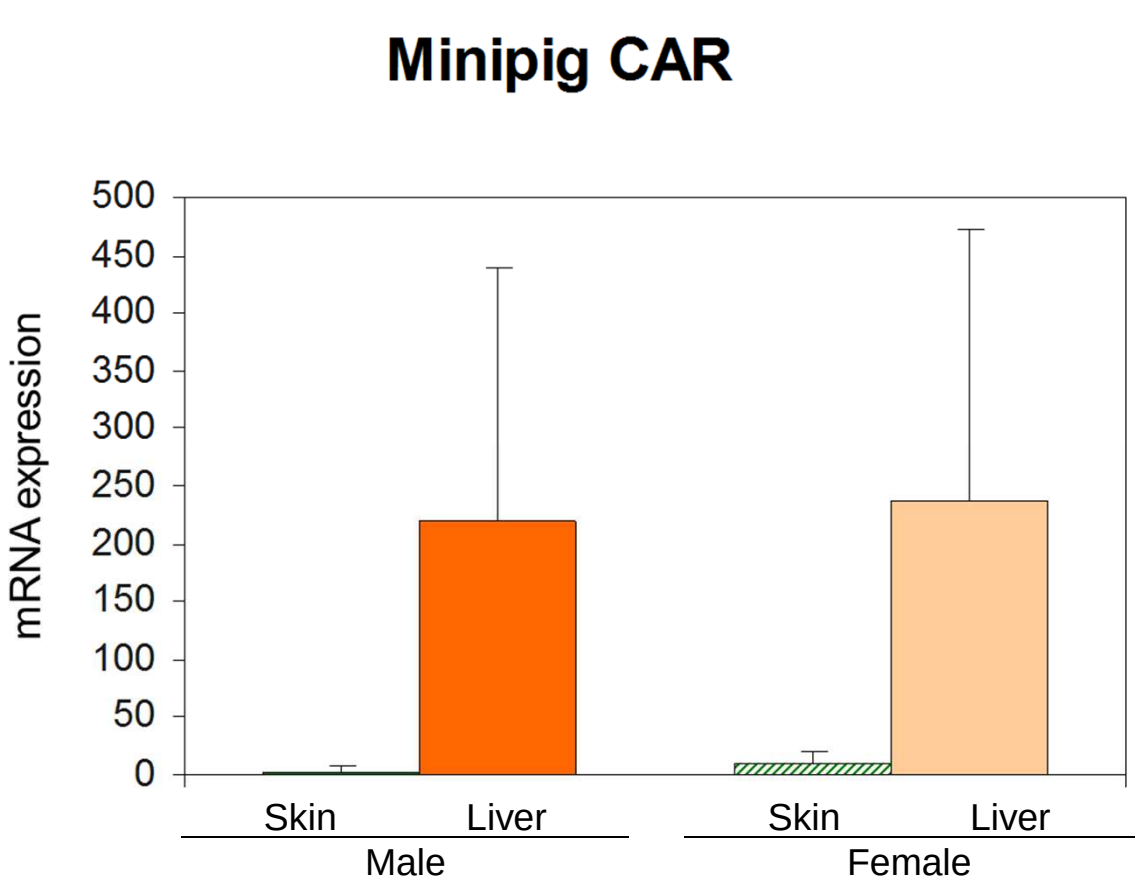
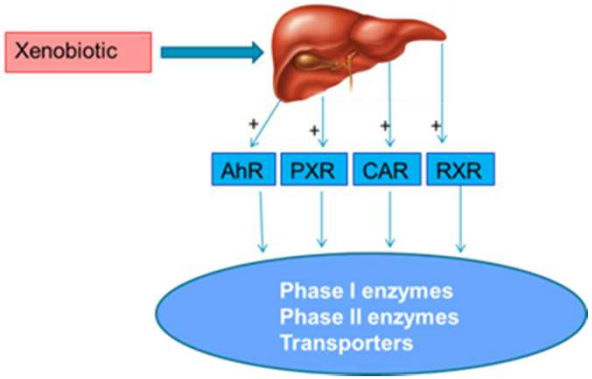
Expression level of CAR in Minipig skin and liver





Very low expression level of CAR in minipig skin compared to liver

Comparison between Human and Minipig

- Liver over skin ratio in human and minipig

	Human		Minipig	
	Male	Female	Male	Female
CYP1A1	53	0.02	2.5	0.58
FMO5	0.24	0.7	82	0.02
CAR	12	13.5	61	23.63

- Expression level is different between male and female and between human and minipig

In vitro Metabolism of Testosterone in Human and minipig

Minipig

Human

LIVER

Microsomes

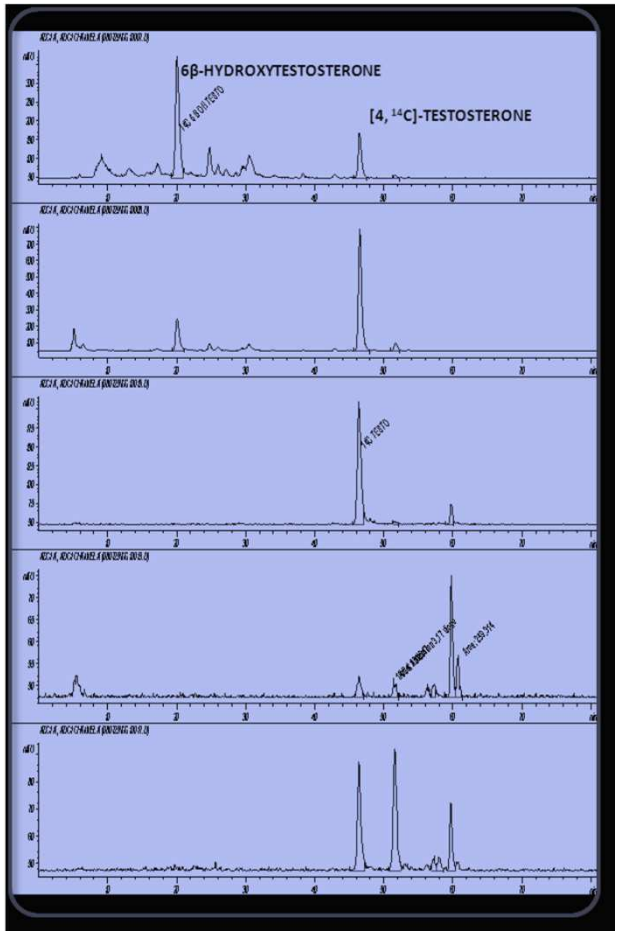
Hepatocytes

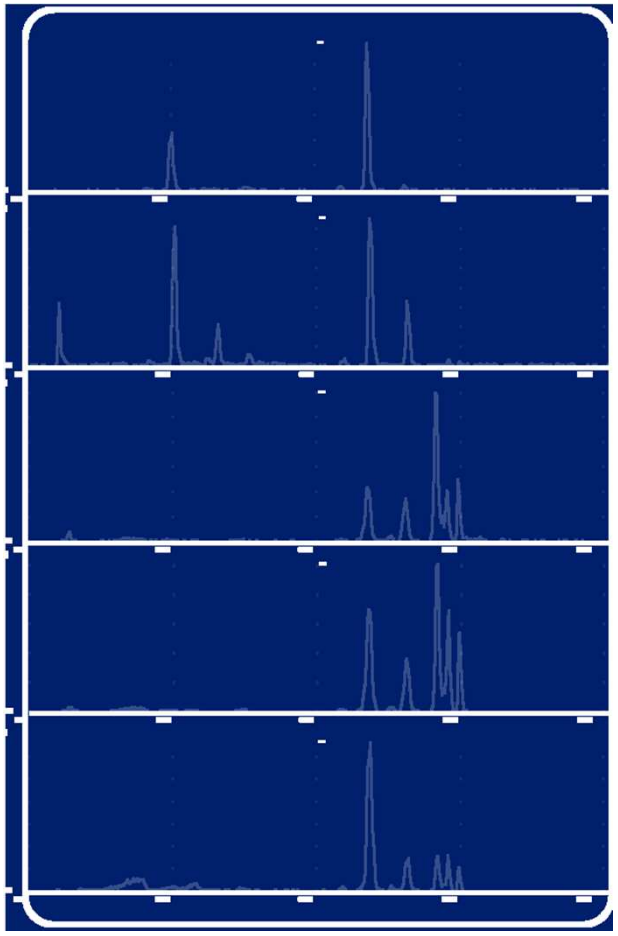
SKIN

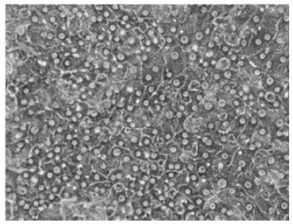
Epidermis

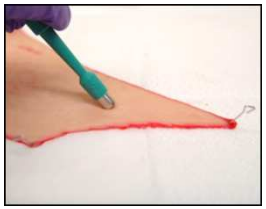
Dermis

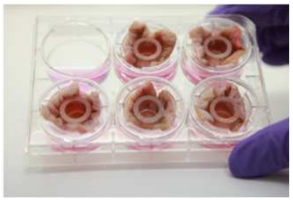
Receptor











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In vitro Metabolism of Testosterone in Human and minipig

- *In vitro* Metabolism of Testosterone is similar in human and minipig,
- *In vitro* Metabolism of Testosterone is different between Skin and Liver

COMPARISON BETWEEN SKIN AND LIVER METABOLISM OF [4, ^{14}C]-TESTOSTERONE IN HUMAN AND MINIPIG

K. Sevin, A. Gaborit, C. Verrier, S. Feyte, P. Comby, B. Ruty, H. Osman-Ponchet
METABOLISM & PHARMACOKINETICS UNIT, PRECLINICAL DEVELOPMENT, GALDERMA R&D, SOPHIA ANTIPOLIS, FRANCE

RELATHIONSHIP BETWEEN SKIN METABOLISM AND SKIN ABSORPTION

H. Osman-Ponchet, C. Verrier, S. Feyte, K. Sevin, A. Gaborit, P. Comby, B. Ruty
METABOLISM & PHARMACOKINETICS UNIT, PRECLINICAL DEVELOPMENT, GALDERMA R&D, SOPHIA ANTIPOLIS, FRANCE

Conclusion

- **Pig skin as an alternative to human skin for skin metabolism studies?**

- Expression of drug metabolizing enzymes was shown in human skin and in minipig skin
  - Some differences were observed between Human and in minipig and between male and female (CYP1A1, FMO5)
  - Metabolism of Testosterone was comparable between minipig and human

- Potential clinical implications and potential drug-drug interaction (limited literature data)

  - Additional data are needed to assess the clinical implication of skin metabolism

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Galderma R&D

Pr. Antoinette Lemoine

Hospital Paul Brousse, INSERM 1004, Paris

Questions?