DEVELOPMENT OF NUCLEAR RECEPTOR TRANSFECTED CACO-2 CELL LINES

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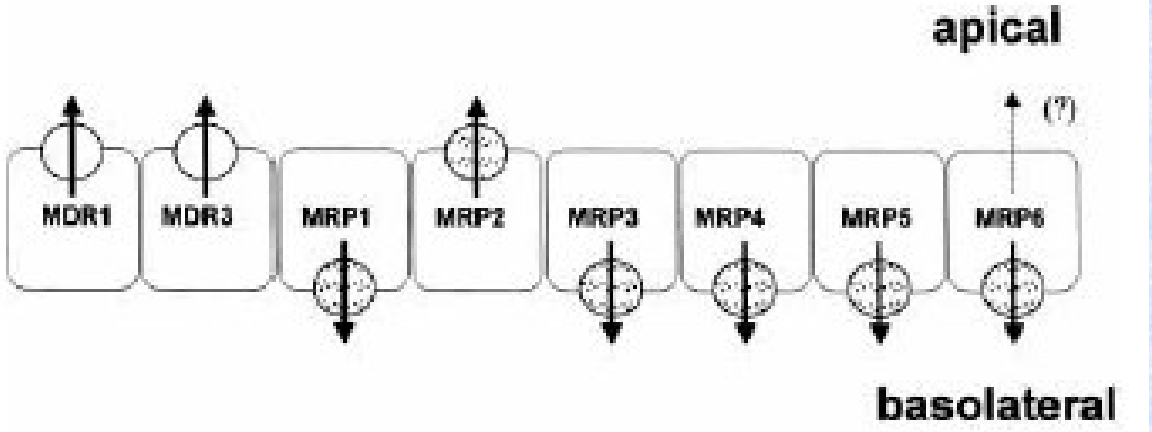
- **Background**
- Cell lines
- Gene expression
- Functional experiments
- Conclusions

Intestinal absorption

- Small intestine is the most important site of drug absorption →predictions in the discovery phase important
- Intestinal epithelium often limits the absorption rate from GIT
- Active first-pass metabolism

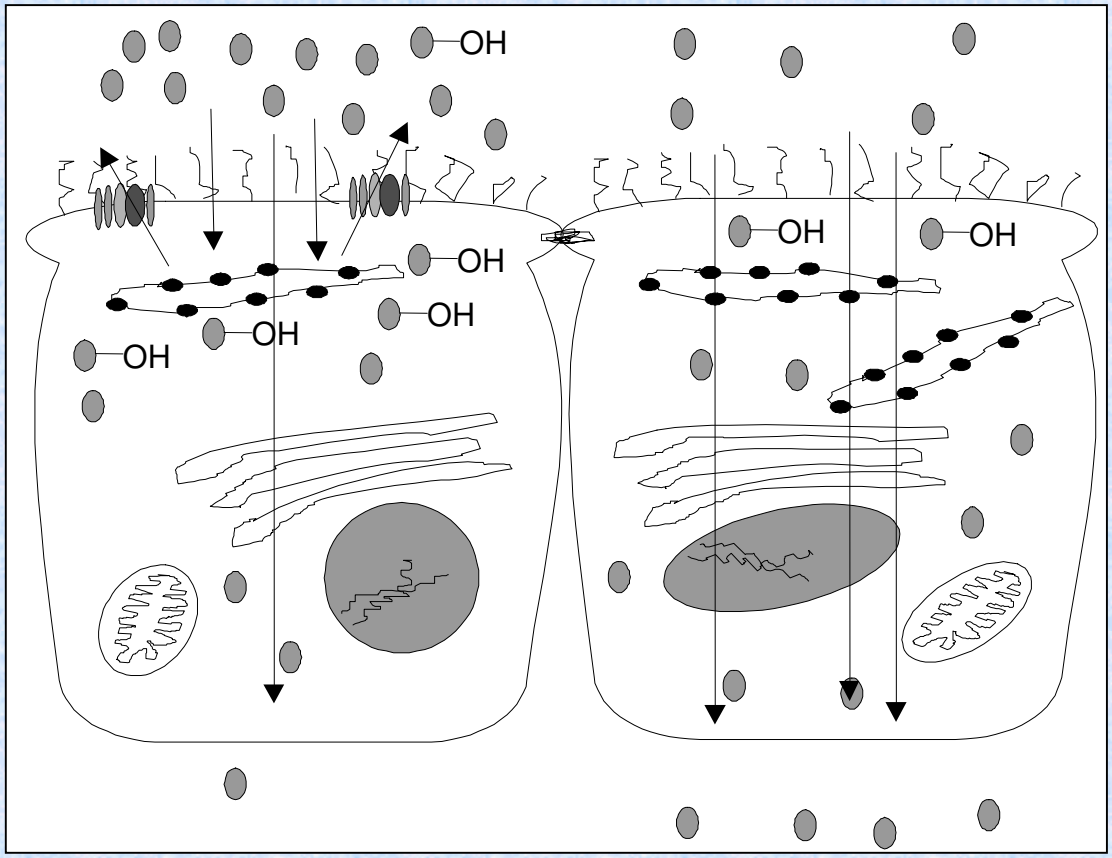
CYPs and efflux pumps

- Cytochrome P450 (CYP) families 1-3 metabolise several xenobiotic compounds in microsomes of several tissues (e.g. liver, small intestine)
- Efflux-pumps (P-glycoprotein, MRP-family, BCRP) excrete several xenobiotic compounds from the cells



Fricker and Miller Pharmacol Toxicol 2002:90:5-13.

CYPs / efflux pumps - interplay



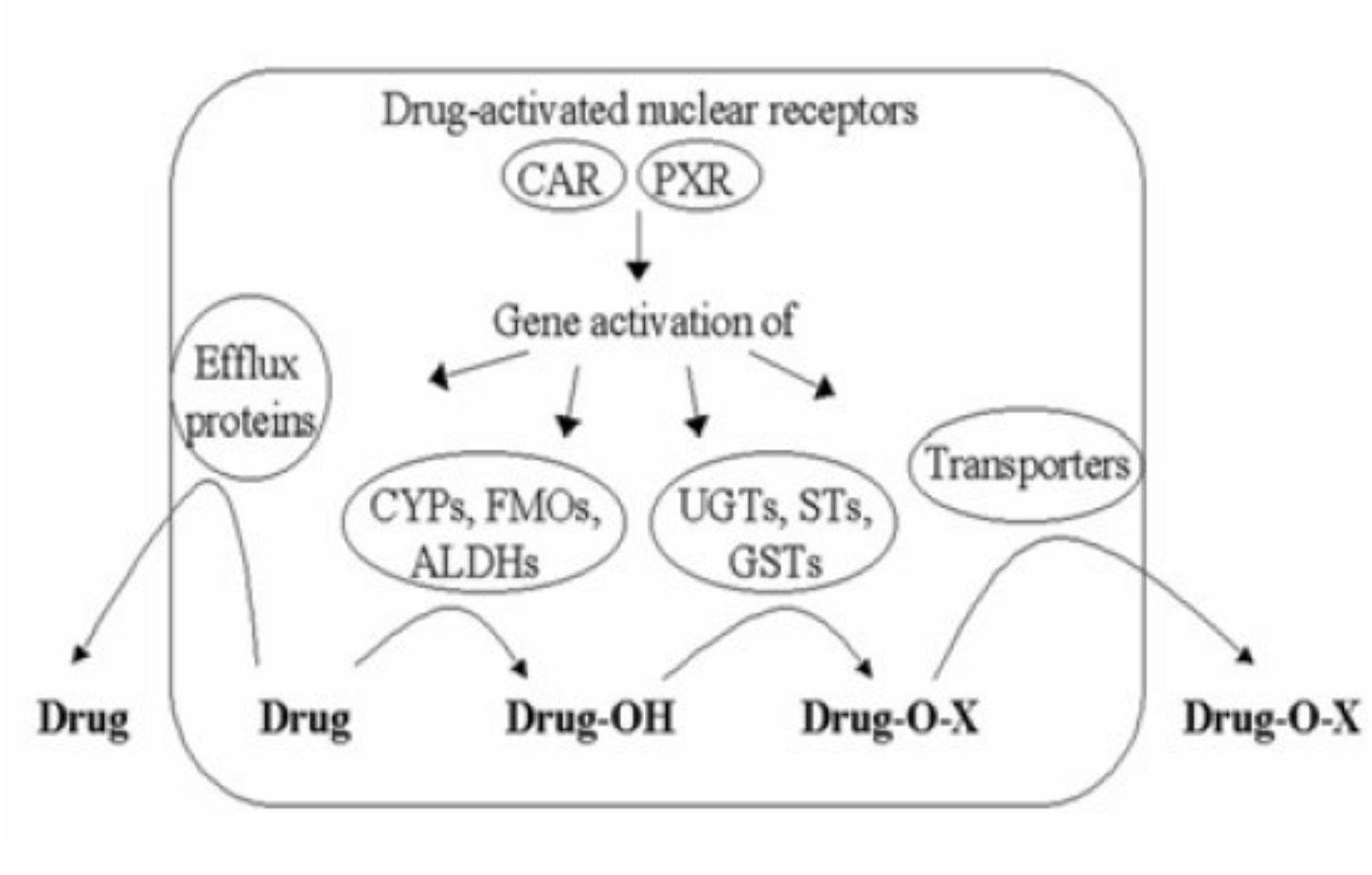
= CYP enzyme

= Efflux pump

= Drug molecule

OH = oxidised drug molecule

Regulation of xenobiotic metabolism - nuclear receptors

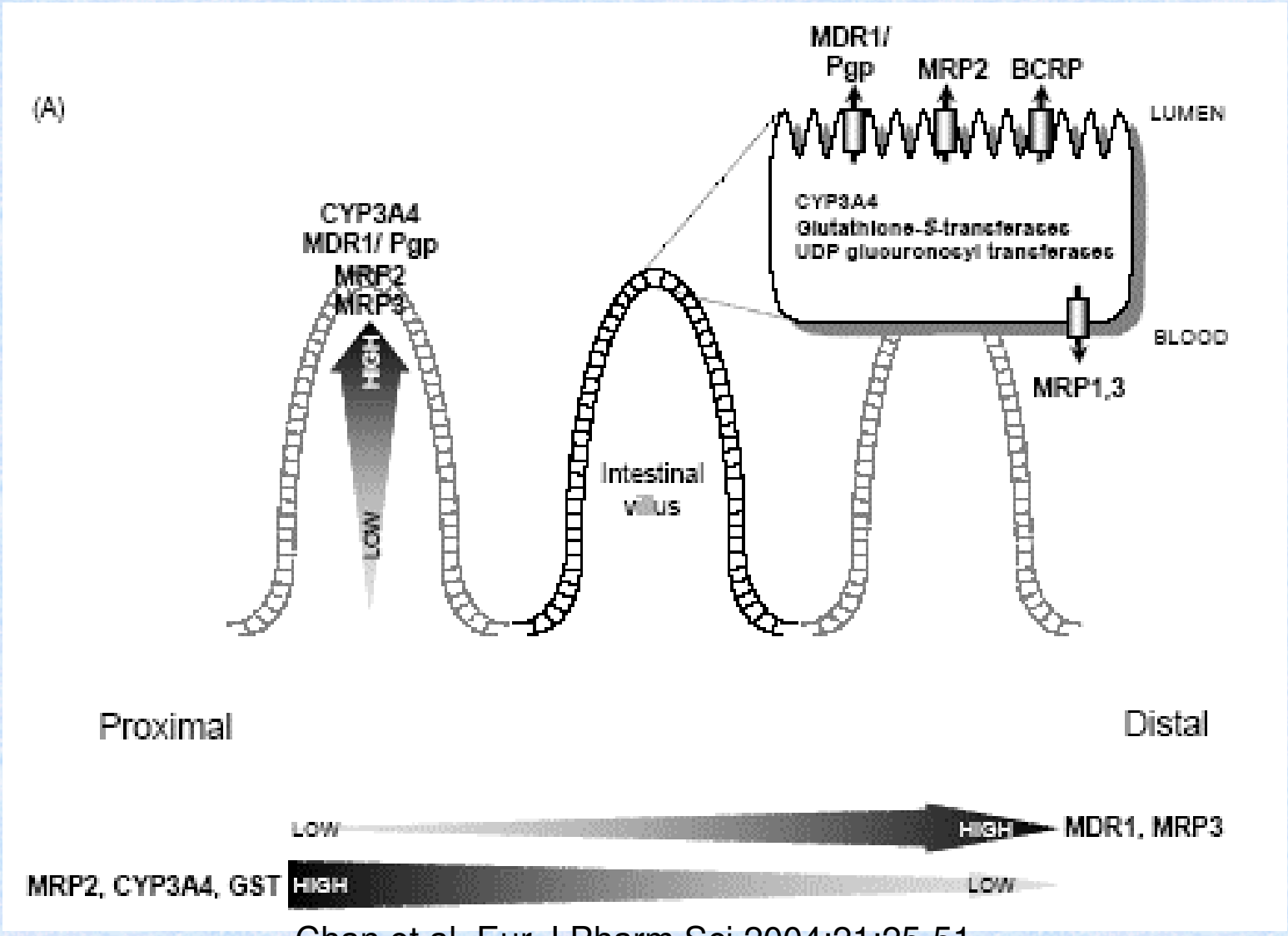


Some target genes:

CYP2B6, CYP2C9, CYP3A4, MDR1

Honkakoski, Current Pharmacogenomics 2003(1):75-85

Average enterocyte?



Chan et al. Eur J Pharm Sci 2004;21:25-51.

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Caco-2 cells

A colon carcinoma cell line that differentiates spontanously into enterocyte-like cells

+ Widely used
+ Human origin
+ Expresses many transporters
+ Relatively easy to grow

- Inter and intralaboratory differences
- Paracellular space very tight
- Incomplete transporter profile
- CYP metabolism absent
- Long growth time

About Caco-2 cells in permeability experiments:

Ungell. Drug Discovery Today Technologies 2004(1): 423-430.

Modified cell lines

Cell line	Modification	Ligands	Some target genes
Caco/WT	Wild type cells		
Caco/hPXR	Transfection with human PXR	+: Rifampicin, ritonavir, hyperforin	**CYP3A4**, **MDR1**, CYP2B6
Caco/mCAR	Transfection with murine CAR	+: TCPOBOP, phenobarbital -: Androstenol, progesterone	**CYP2B6**, MDR1, CYP3A4, CYP2C9, MRP2

Initial characterisation:

T. Korjamo, P. Honkakoski, M. R. Toppinen, S. Niva, M. Reinisalo, J. J. Palmgren, and J. Monkkonen. Absorption properties and P-glycoprotein activity of modified Caco-2 cell lines. Eur J Pharm Sci, 26:266-279 (2005).

Induction properties:

T. Korjamo, J. Monkkonen, J. Uusitalo, M. Turpeinen, O. Pelkonen, and P. Honkakoski. Metabolic and efflux properties of caco-2 cells stably transfected with nuclear receptors. Pharm Res, 23:1991-2001 (2006).

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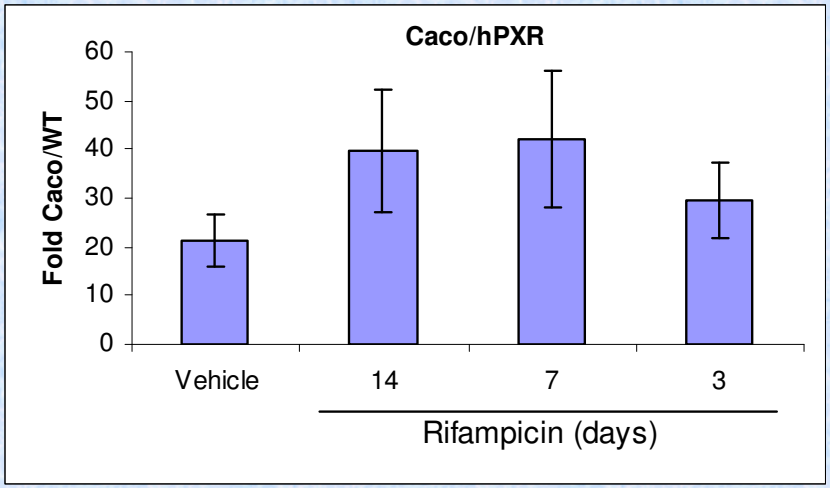
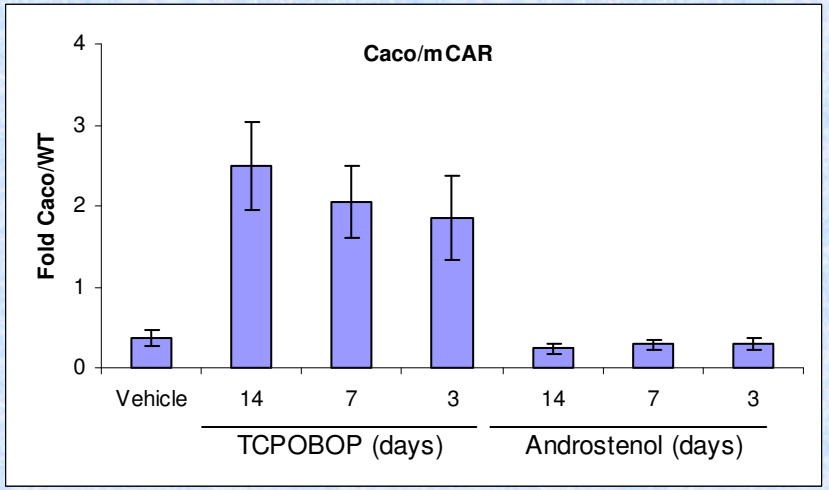
Changes in transcription: qRT-PCR

R = hPXR activator rifampicin, T = mCAR activator TCPOBOP, A = mCAR inhibitor androstenol

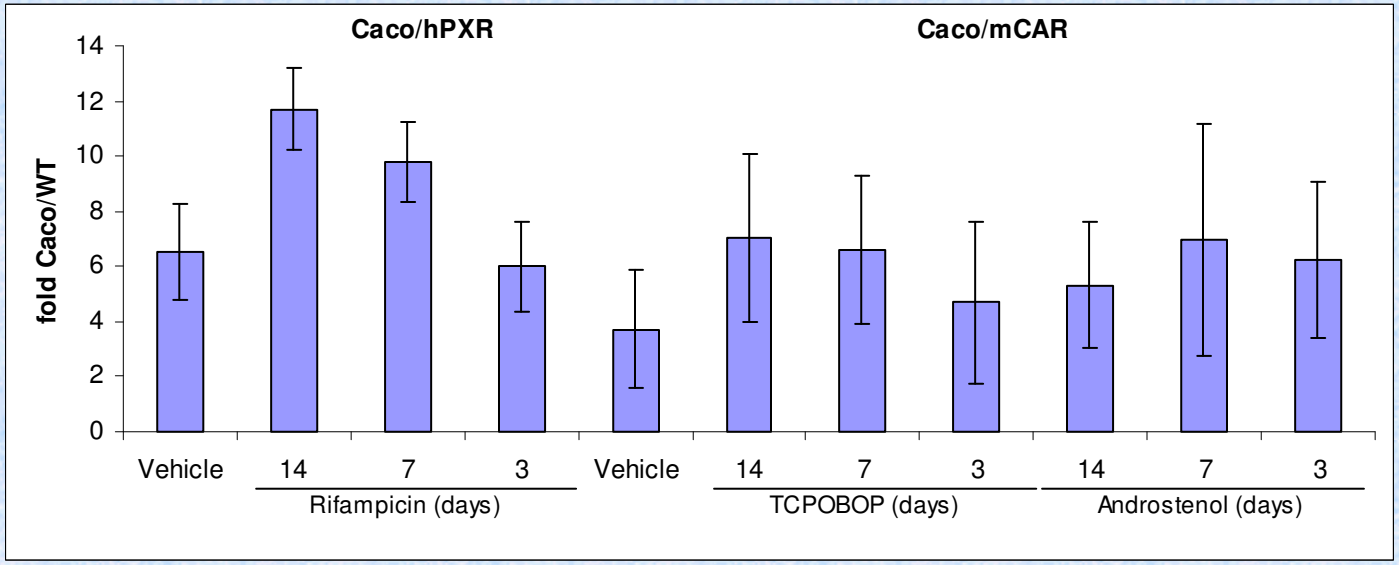
CYP3A4



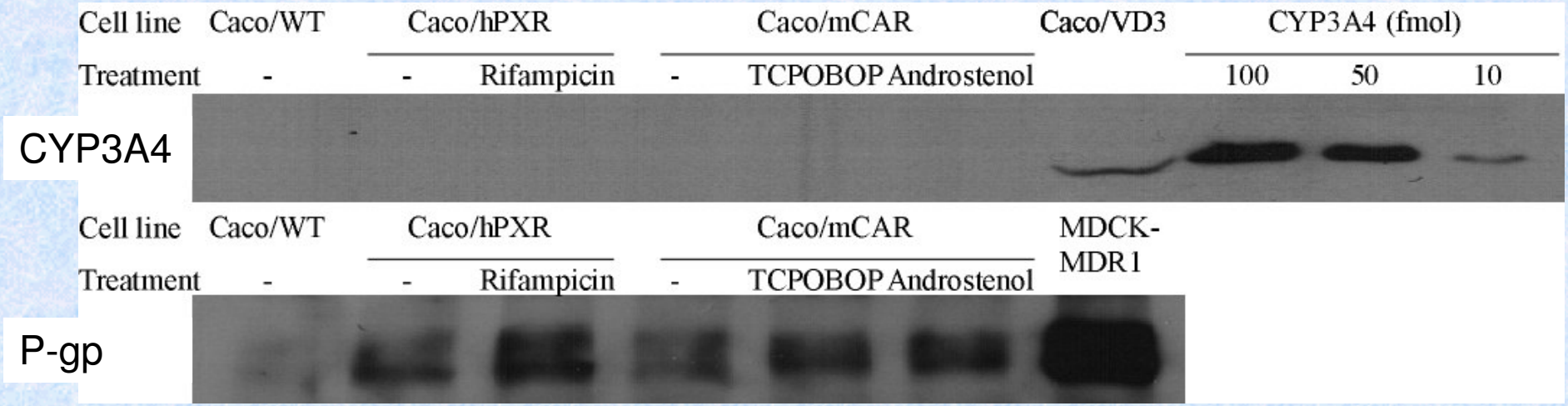
CYP2B6



MDR1



Protein level



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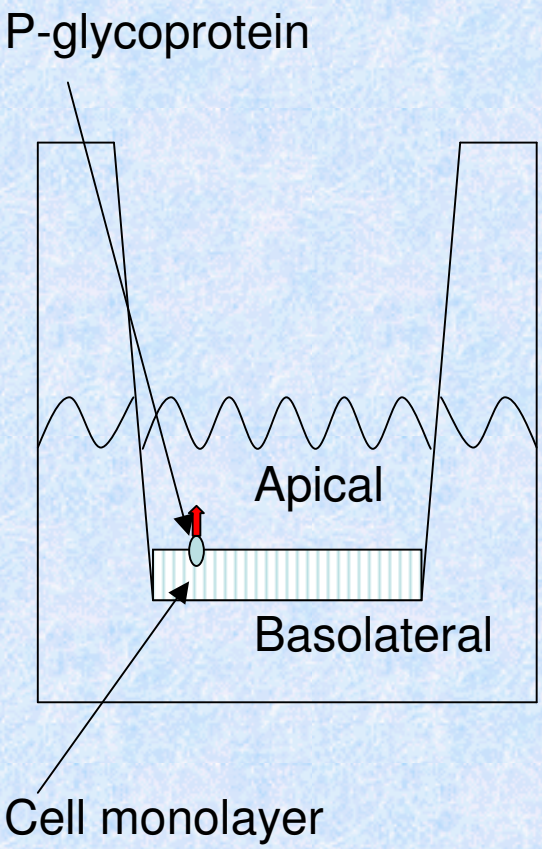
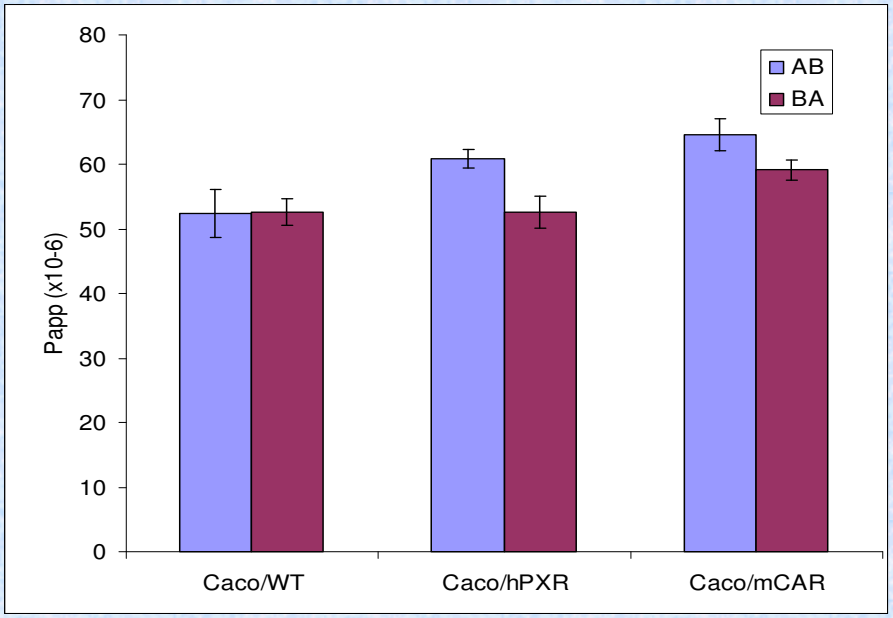
Permeability experiments



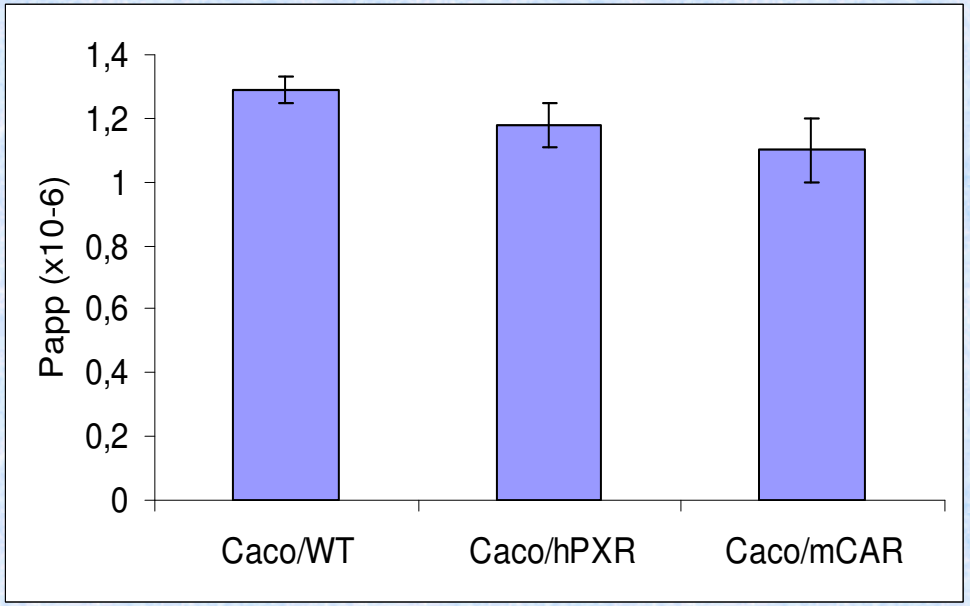
- Apical to basolateral (AB): "from intestinal lumen to circulation"
- Basolateral to apical (BA): "from circulation to intestinal lumen"
- $P_{AB}>P_{BA}$ -> Active absorption
- $P_{AB}<P_{BA}$ -> Efflux pump (active secretion)
- pH-gradients may cause deviations if ionisable molecules are studied!

Passive permeability

Antipyrine (transcellular)

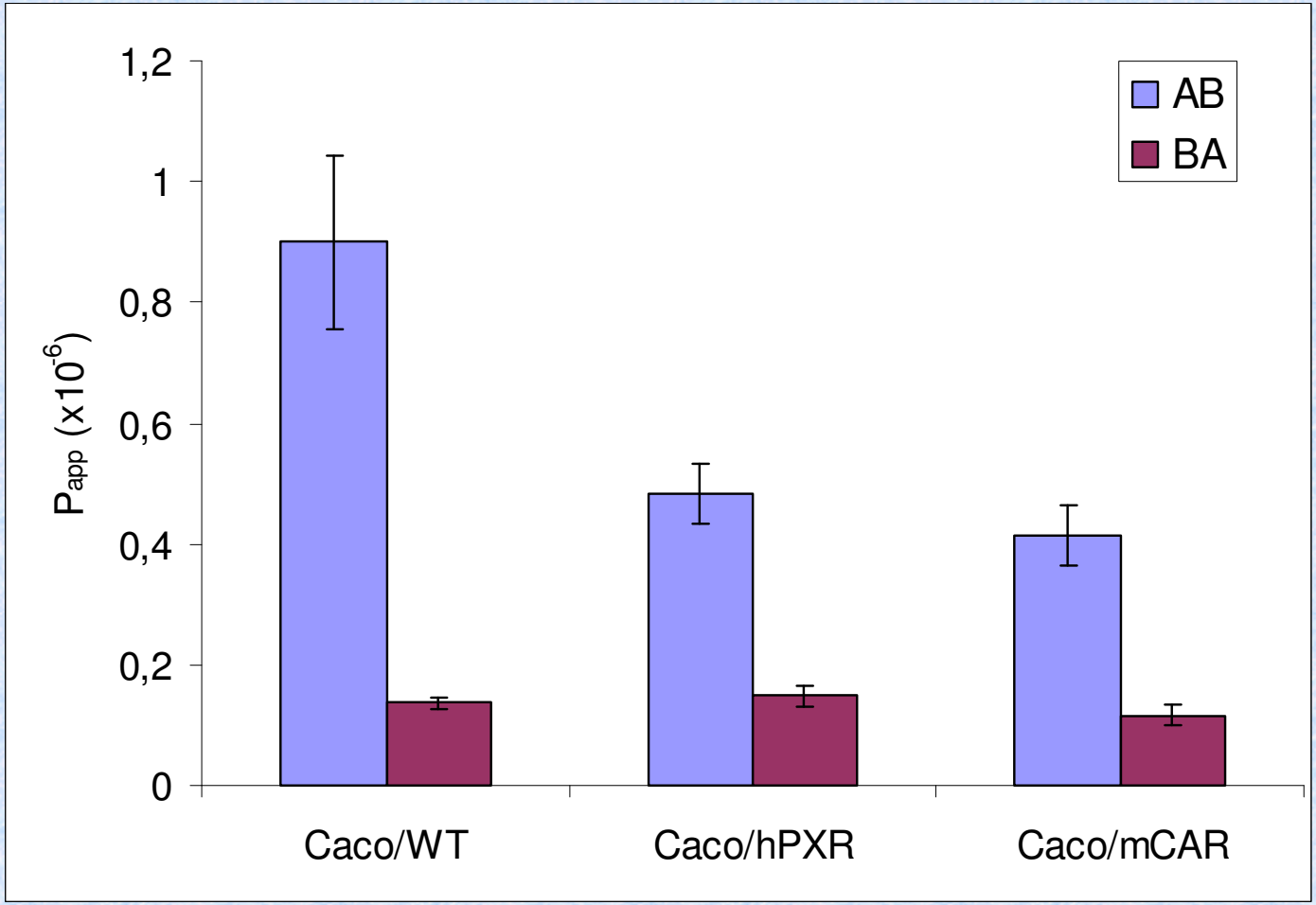


Mannitol (paracellular)

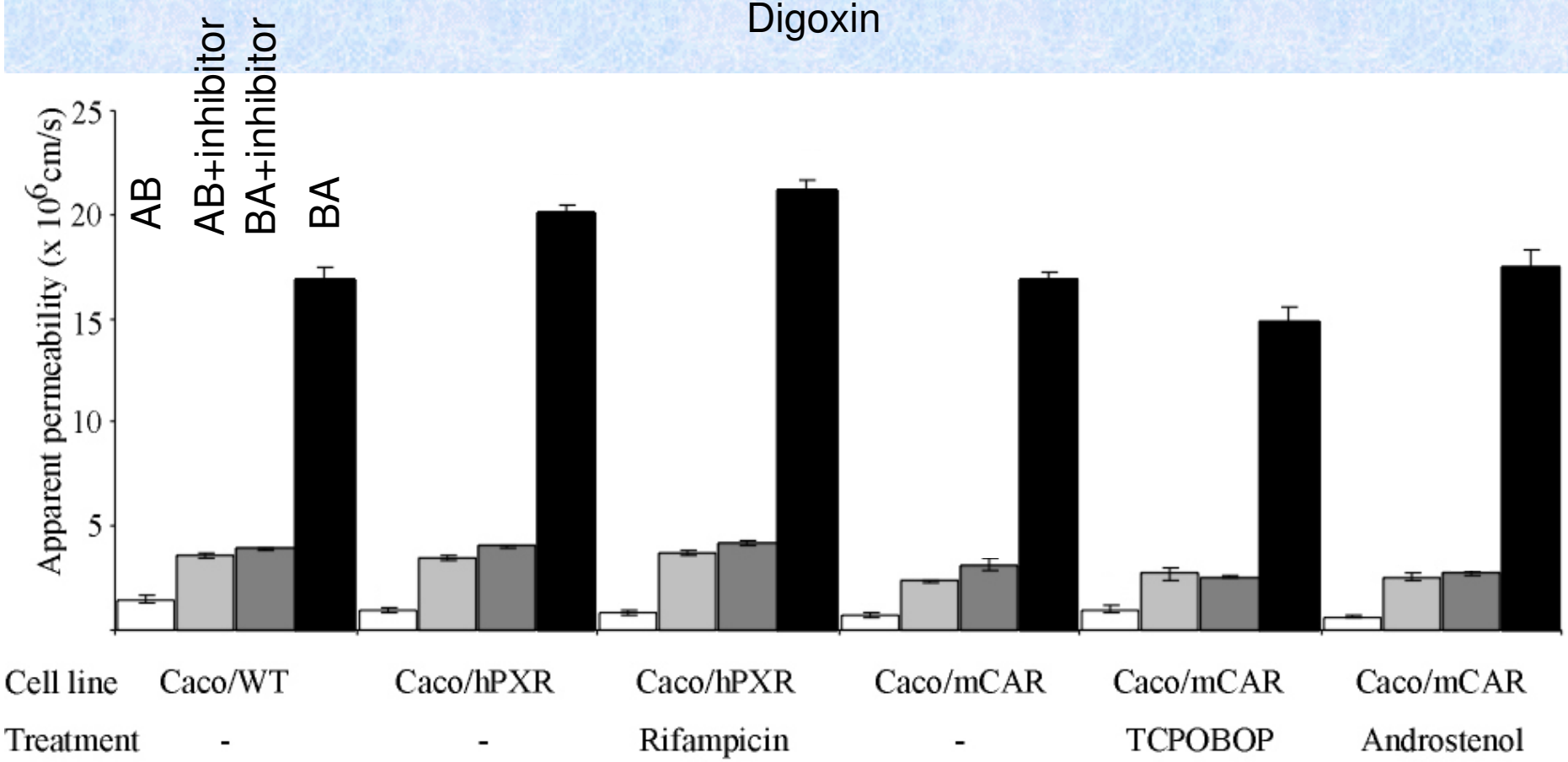


Active absorption

Cephalexin (hPepT1)



Permeability involving P-gp



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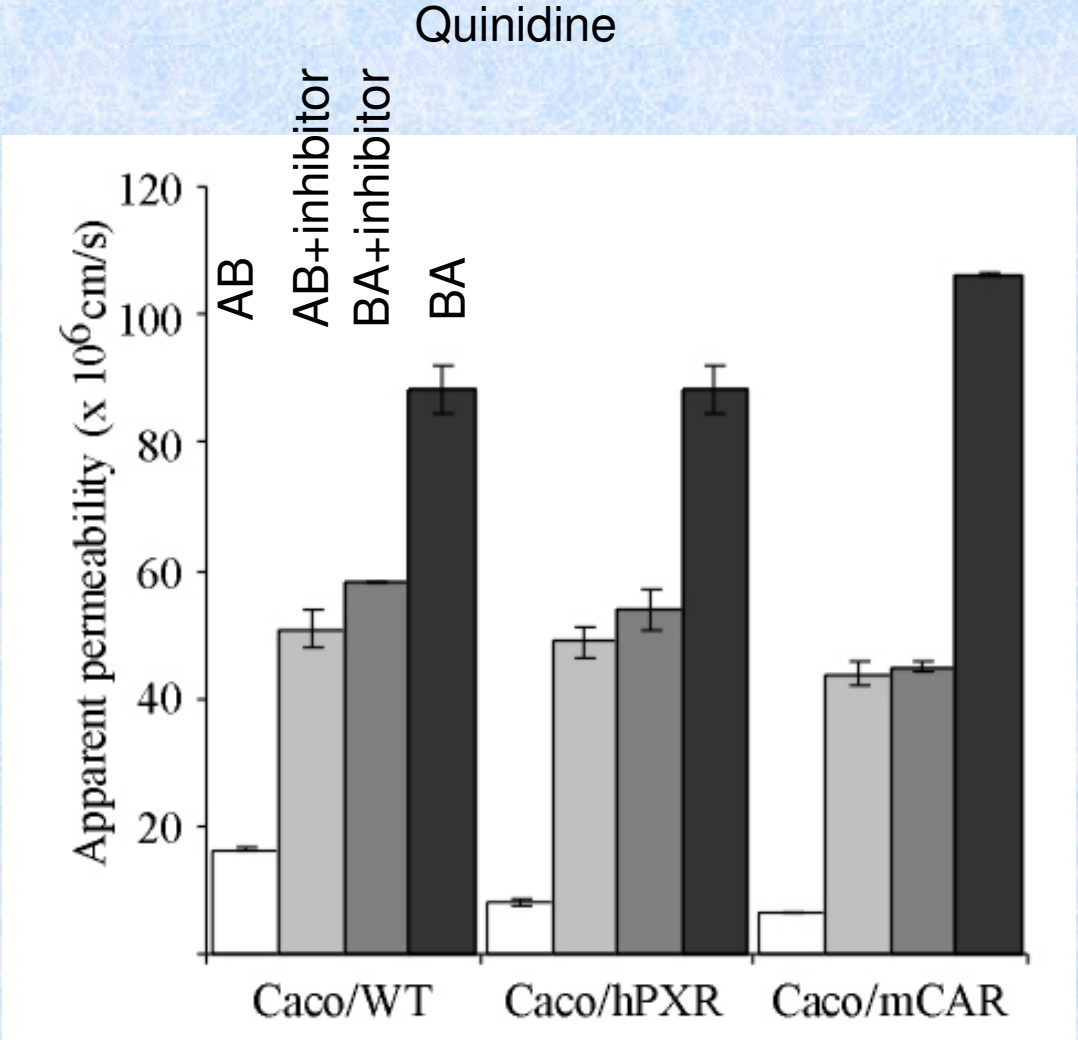
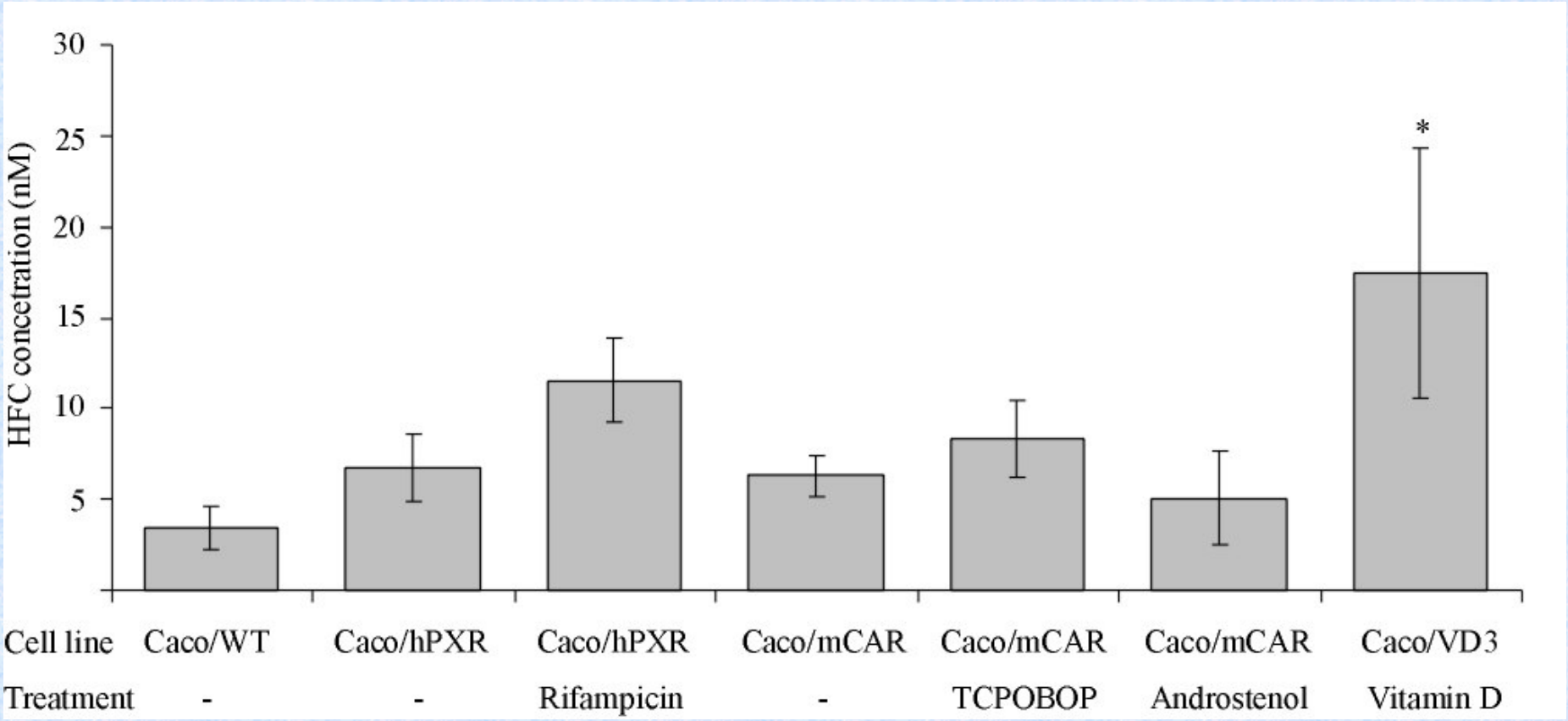


CYP3A4 mediated metabolism

Ketoconazole sensitive HFC formation from BFC



Conclusions

- Stable transfectants retain viability and passive transport properties
- Genes can be controlled in Caco-2 cells with nuclear receptors and their ligands
- P-gp induction can also be seen at protein and functional levels
- CYP3A4 protein level and activity remain very modest

Acknowledgements

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