Use of *in vitro* cell assays and noninvasive imaging techniques to reduce animal experiments in drug development

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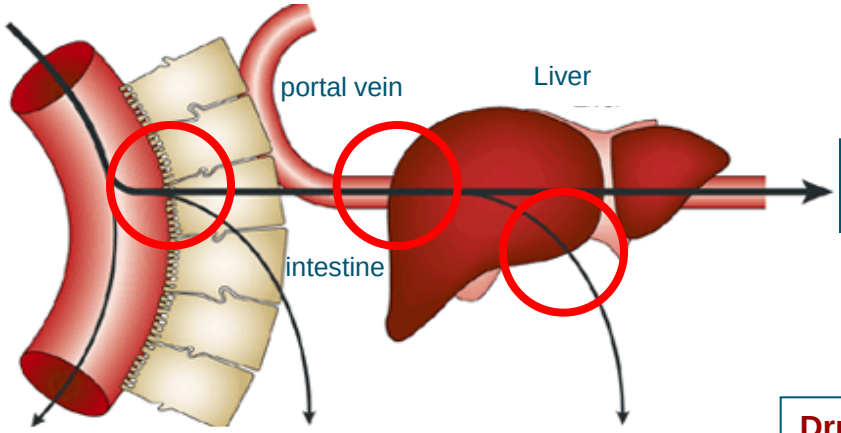
distribution

elimination

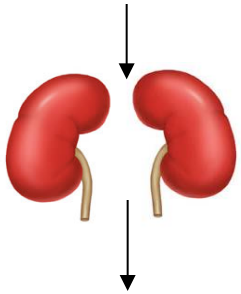
absorption

metabolization



Bioavailability

Drug efficacy and side effect



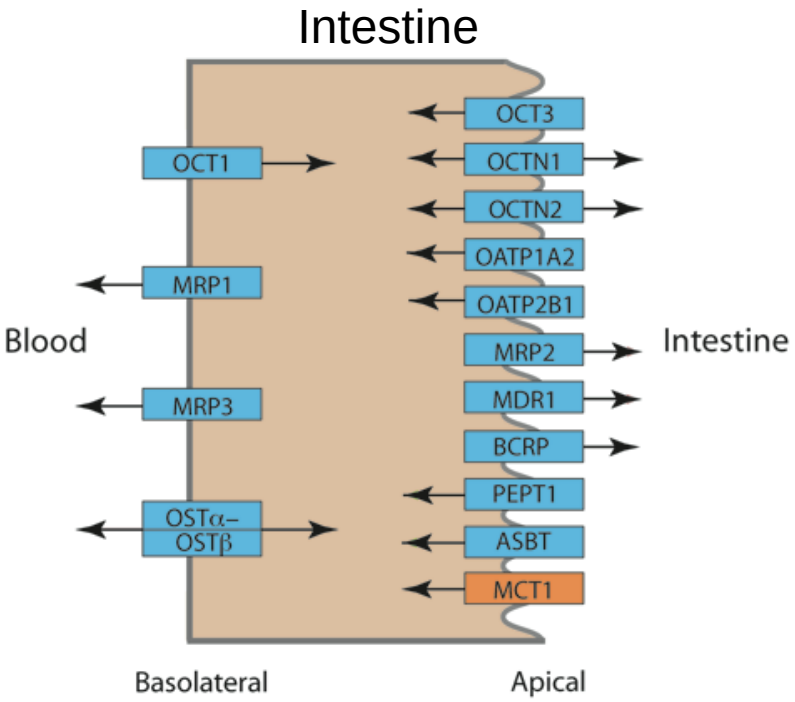
Renale
elimination

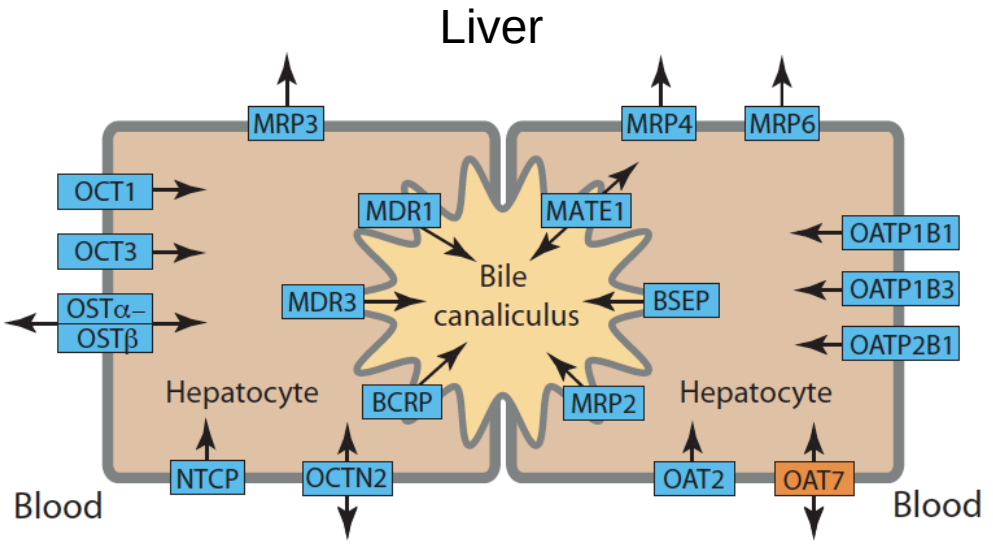
Membrane transporters

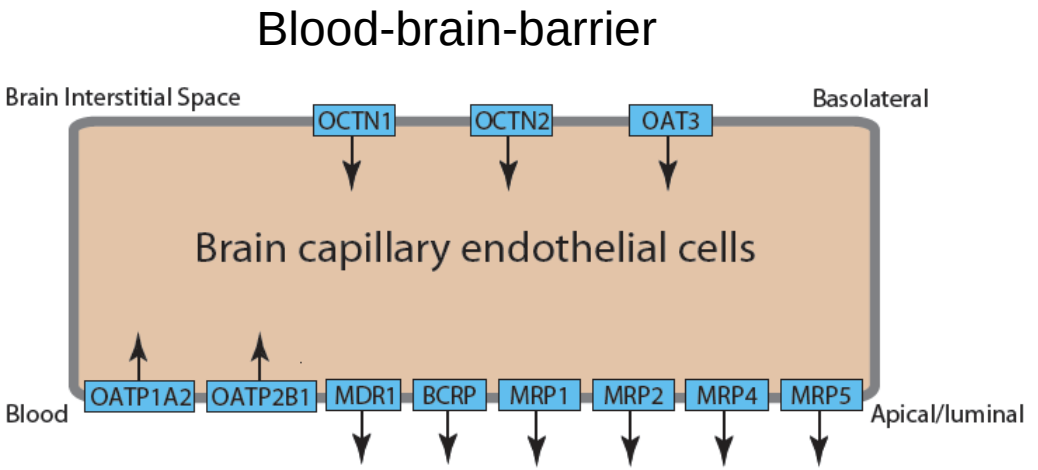
- can be major determinants of the pharmacokinetic, safety and efficacy profiles of drugs

- two major superfamilies — ATP-binding cassette (ABC) and solute carrier (SLC)

Expression of membrane transporters

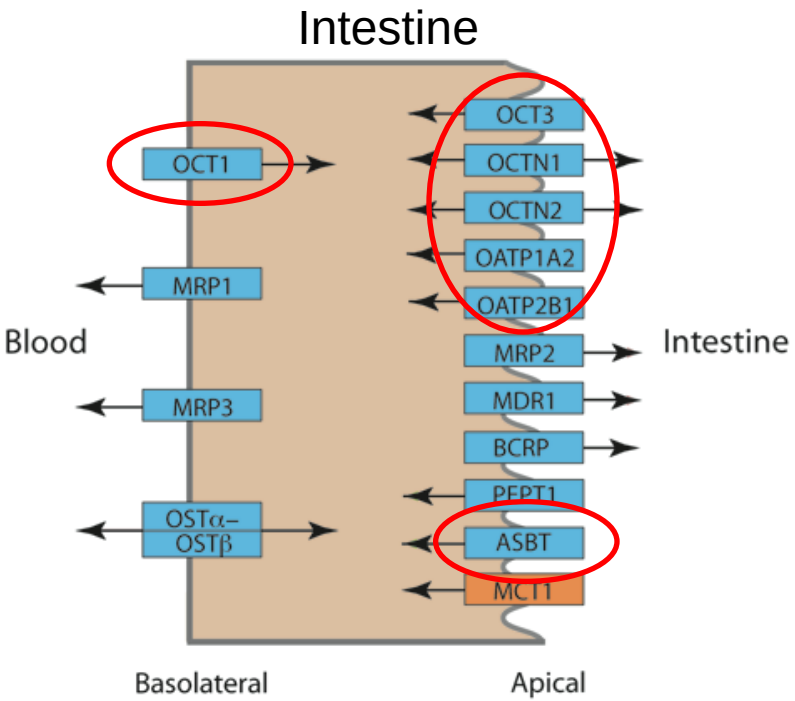


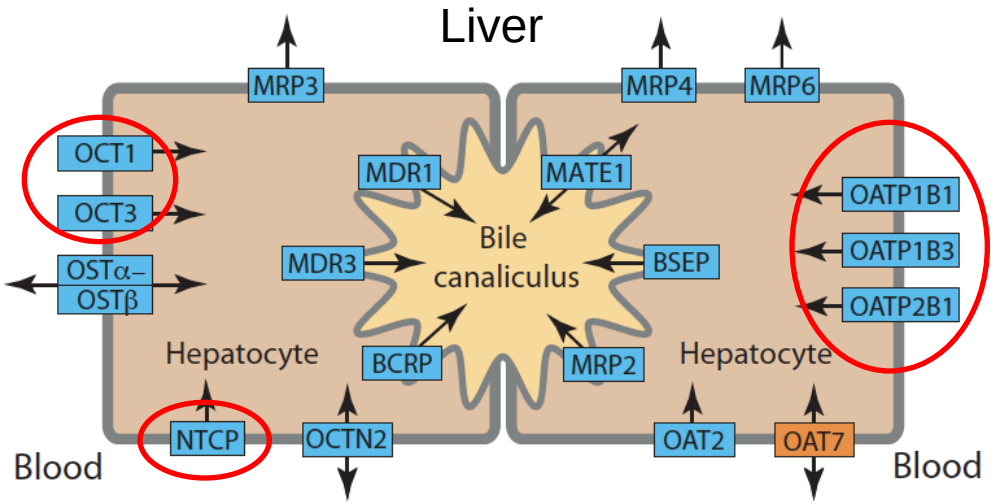


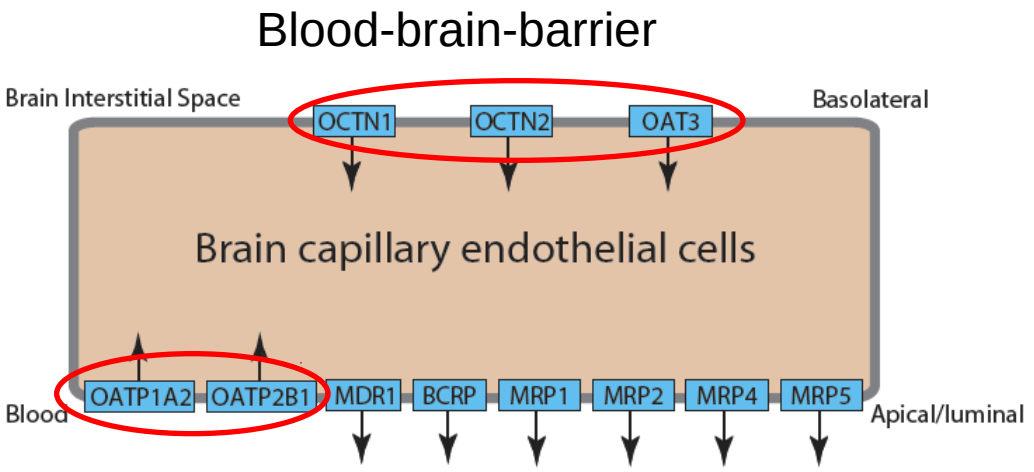


International Transporter Consortium, Nat Rev Drug Discov. 2010

Uptake transporters

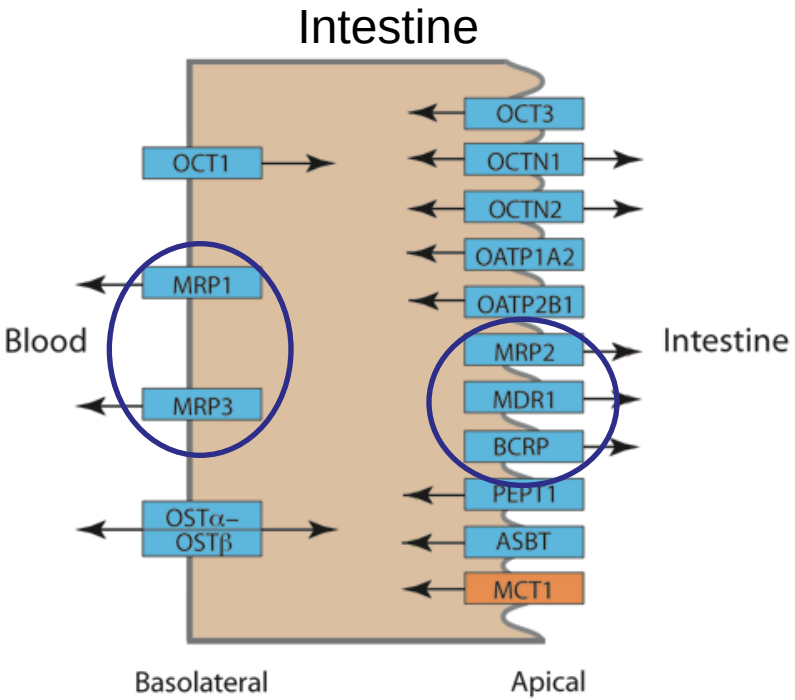


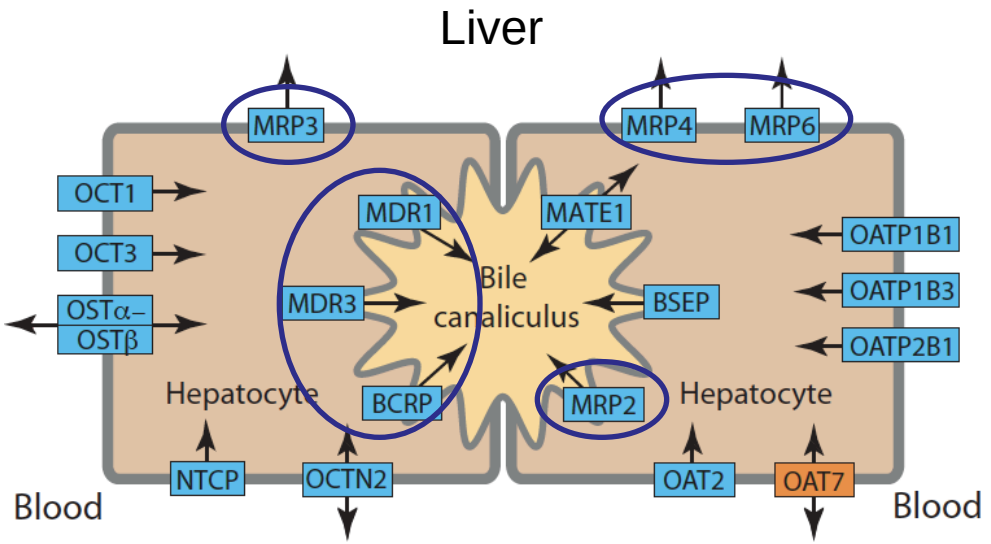


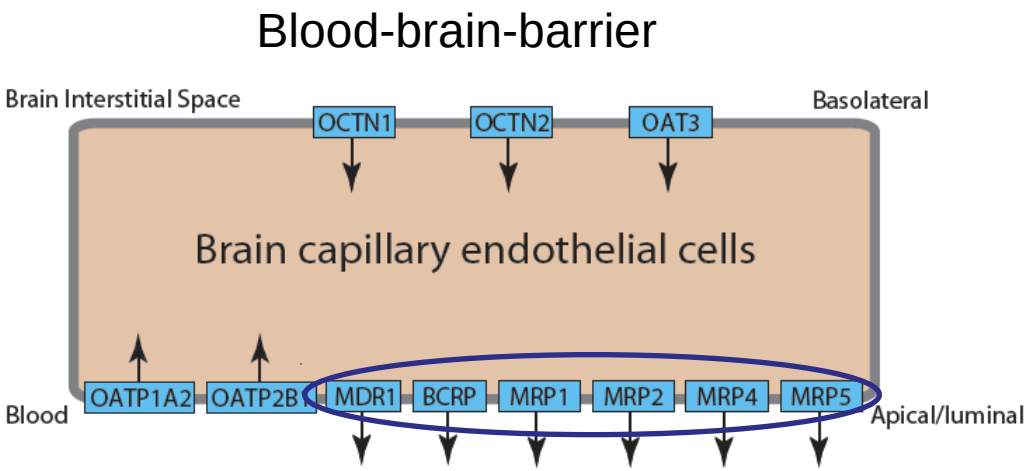


International Transporter Consortium, Nat Rev Drug Discov. 2010

Efflux transporters







International Transporter Consortium, Nat Rev Drug Discov. 2010

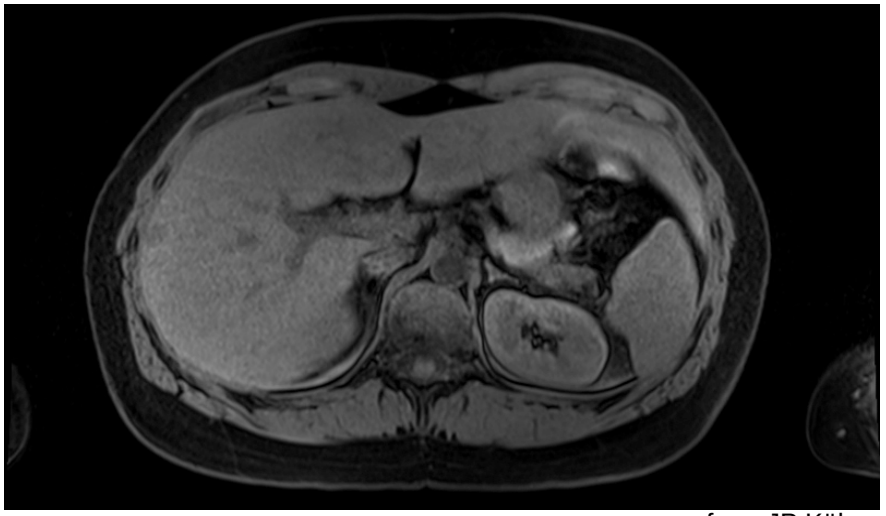
Probe drugs

- The function of drug transporters can be explained by the use of probe drugs

Transporterprotein	***Probe drugs***
P-glycoprotein	Verapamil, Talinolol, Digoxin
OATP1B1	Pravastatin

- Associated with the organ removal from experimental animals

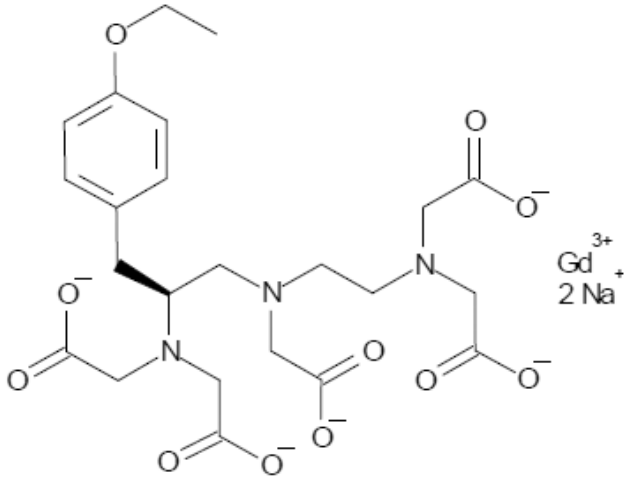
Magnetic resonance imaging (MRI)

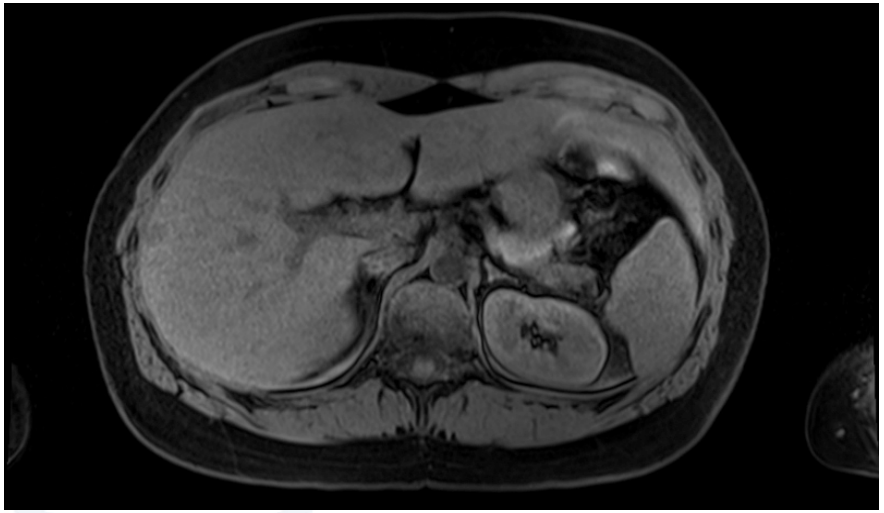


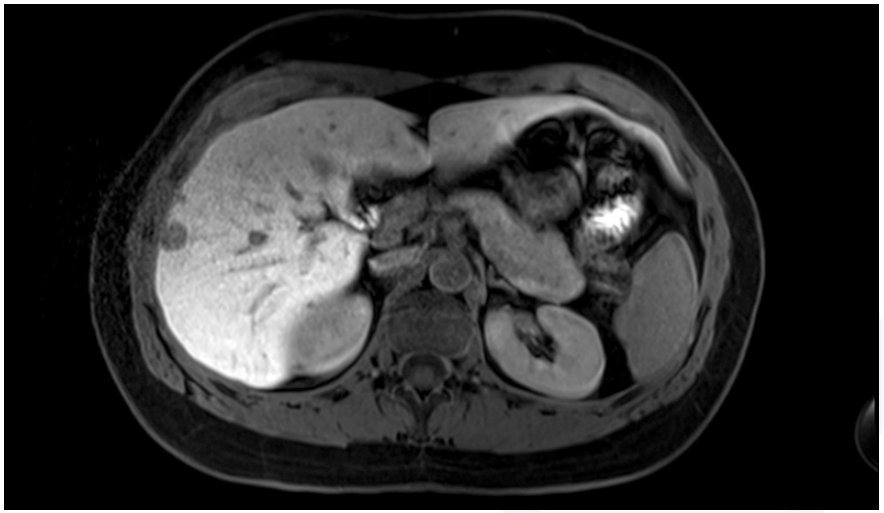
from JP Kühn

Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA, Primovist®)

- Gadolinium-based MRI- contrast agent
- significantly improves detection and characterization of focal liver lesion
- selektivly taken up in the liver cells







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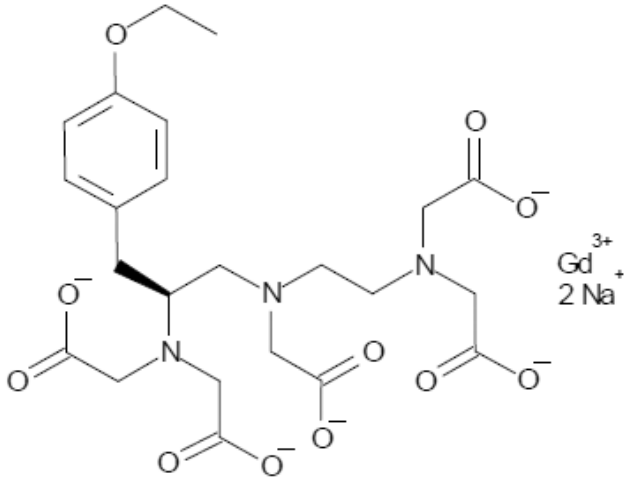
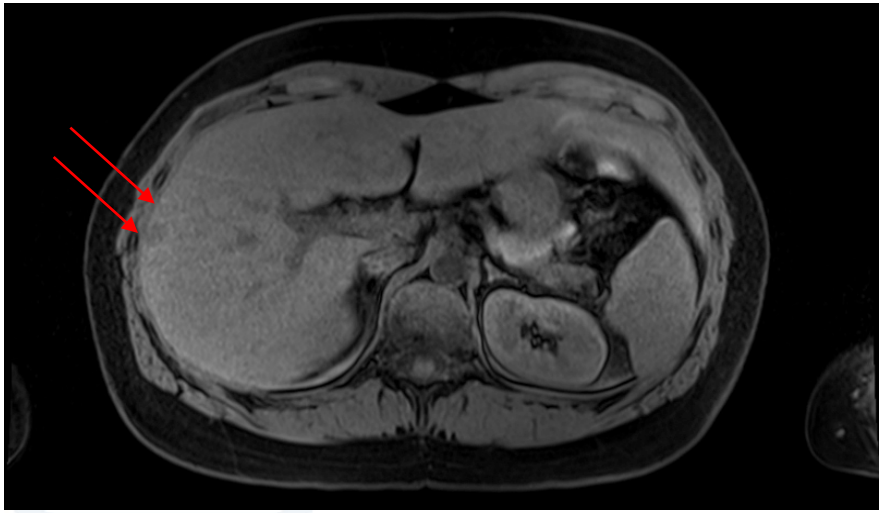
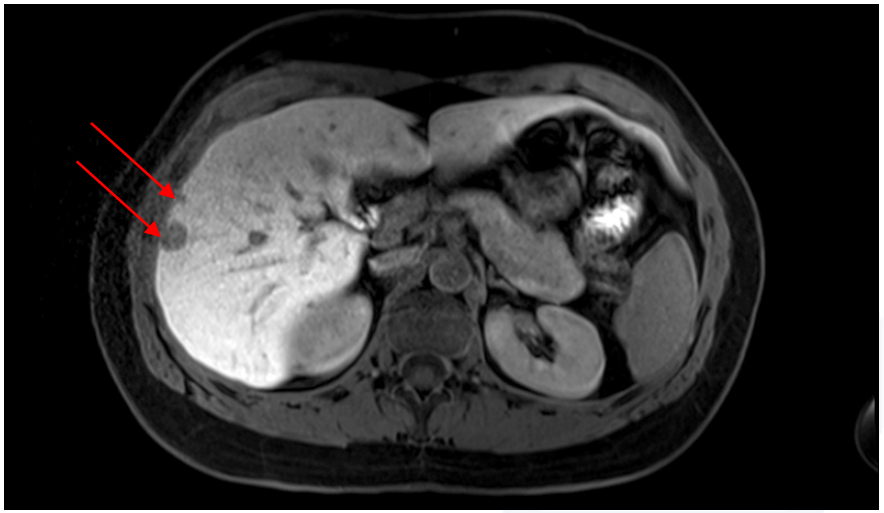


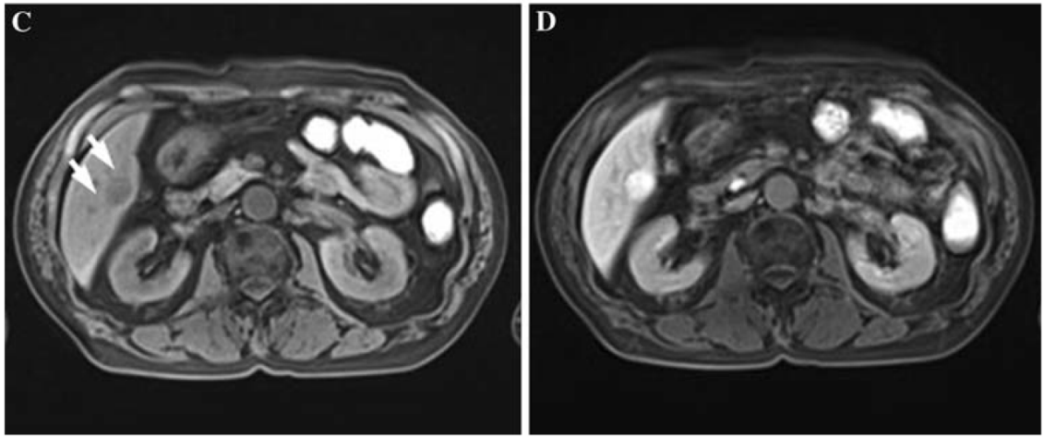


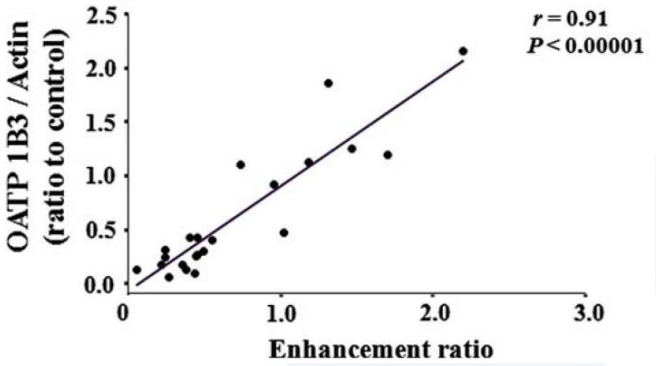


from JP Kühn

Evidences for Gd-EOB-DTPA (Primovist®) to be a substrate of hepatic transporters

- substrate of rat Oatp1a1 in Xenopus laevis oocytes
- known inhibitors of Oatps (BSP, rifampicin) compete with the hepatic enhancement in rodents (van Montfoort et al. 1999)
- enhancement in hepatocellular carcinoma tissue is predicted by expression of human OATP1B3 (Narita et al. 2009)





Narita et al., J Gastroenterol 2009

Hypothesis

- Gd-EOB-DTPA (Primovist®) as a new probe drug
- To visualize and characterise the function of transporter proteins and the drug absorption
  - cellular uptake and elinimation via the same transporters like many drugs

Purpose

❖ *In vitro*:

➢ Identify the transporters of Gd-EOB-DTPA (Primovist®) for the hepatic and intestinal uptake and elimination

❖ *In vivo:*

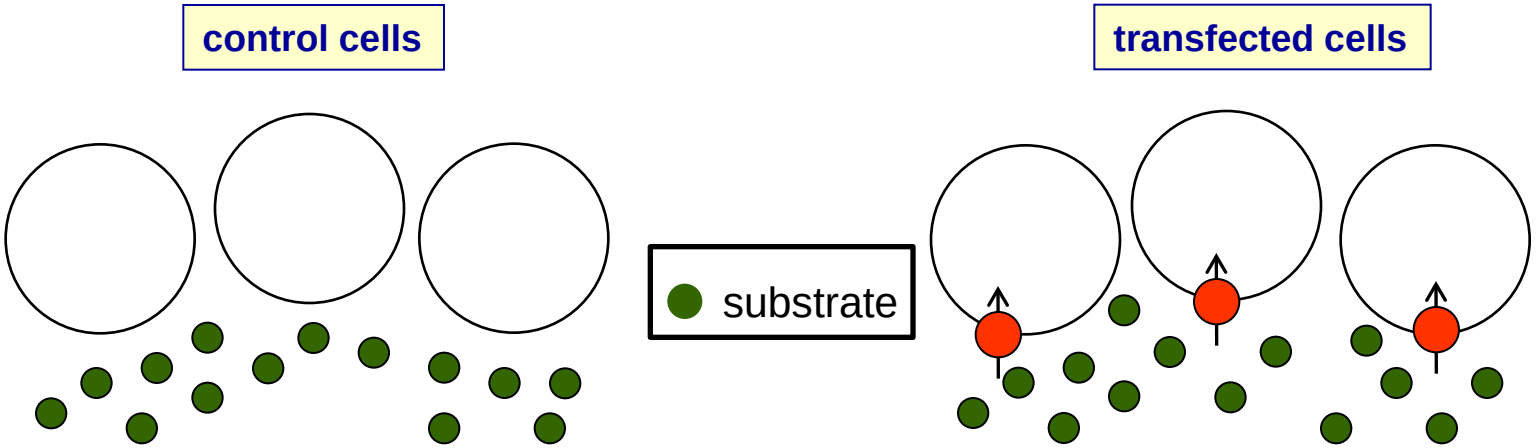
➢ Phamacokinetics (i.v. und oral) and MRI analysis with wild-type and Mrp2-deficient rats

➢ Reduce the number of experimental animals

✓ Gd-EOB-DTPA (Primovist®) in liver can be quantified using MRI without removal of tissue samples from experimental animals

In vitro method to analyze the substrate affinity to uptake transporters

Uptake assay

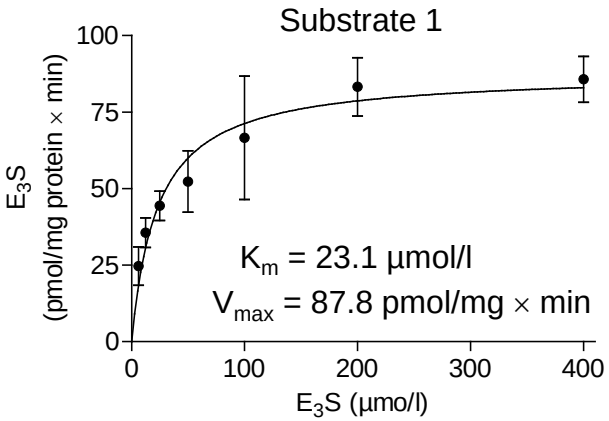


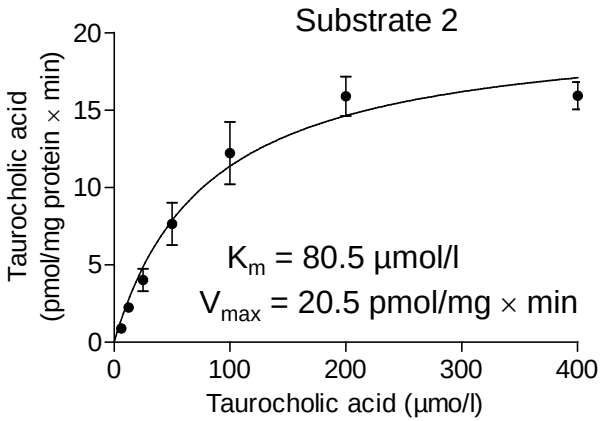
Stable transfected cell lines in the C_DAT

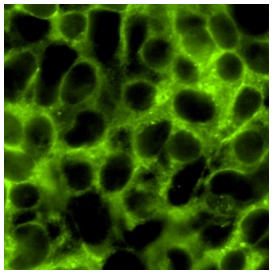
HEK293-cells (human embrionic kidney)		MDCK2-cells (Madin-Darby canine kidney)	
OATP1A2	*1	OATP1A2	*1
	*2		*2
	*3		*3
OATP1B1	*1a	OATP1B1	*1a
	*1b		*1b
	*5		*5
	*15		*15
OATP1B3	WT	OATP1B3	
	c.334T>G		
	c.699G>A		
	c.1564G>T		
	c.334T>G + 699G>A		
OATP2B1	WT	OATP2B1	WT
	c.601G>A		c.601G>A
	c.995G>A		c.995G>A
	c.1457C>T		c.1457C>T
OATP1C1			
OATP3A1		OATP3A1	
OATP4A1		OATP4A1	
OATP4C1		OATP4C1	
OCT1		OCT1	
OCT2		OCT2	
OCT3		OCT3	
OCTN2		OCTN2	
NTCP		NTCP	
ASBT		ASBT	
ABCB1		ABCB1	
ABCC2		ABCC2	
		ABCC3	

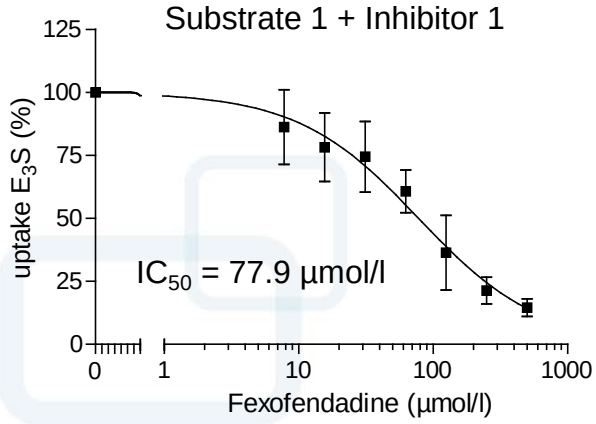
Characterization of stable transfected cell lines

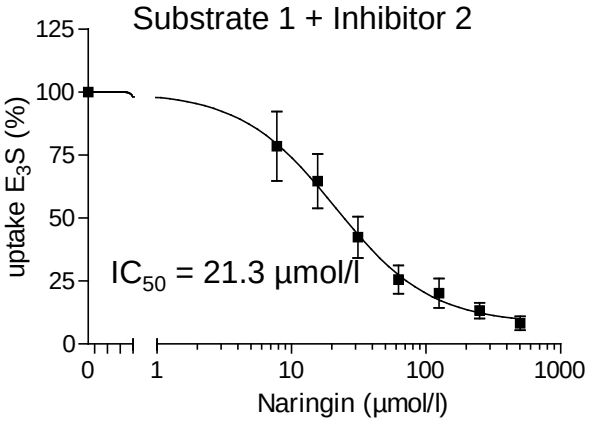
HEK-OATP1A2

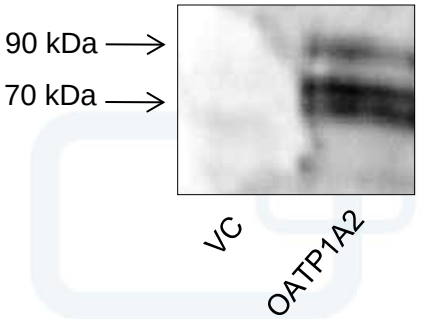




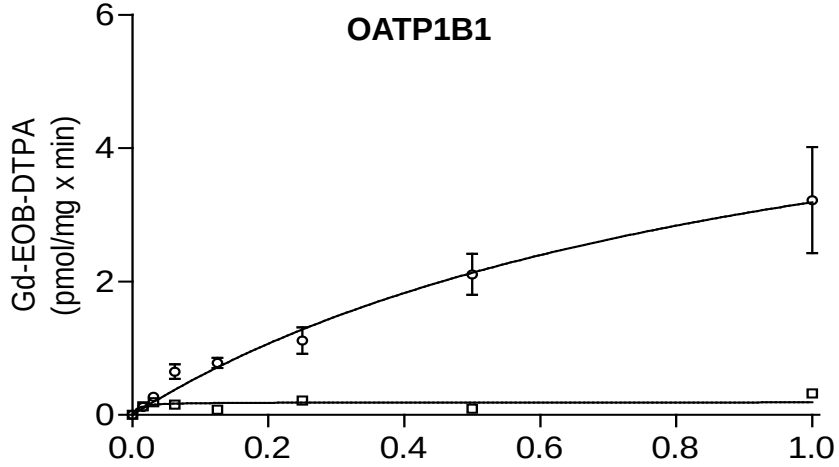


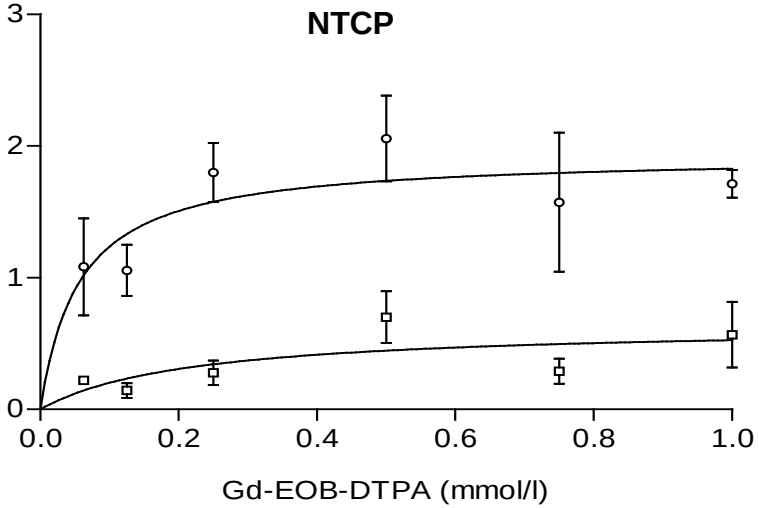


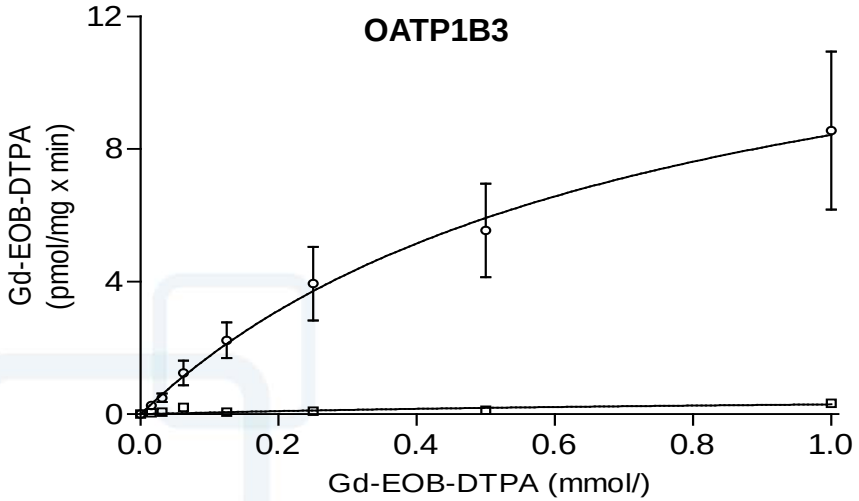




Affinity of Gd-EOB-DTPA (Primovist®) to uptake transporters







Leonhardt et al., Drug Metab Disp 2010

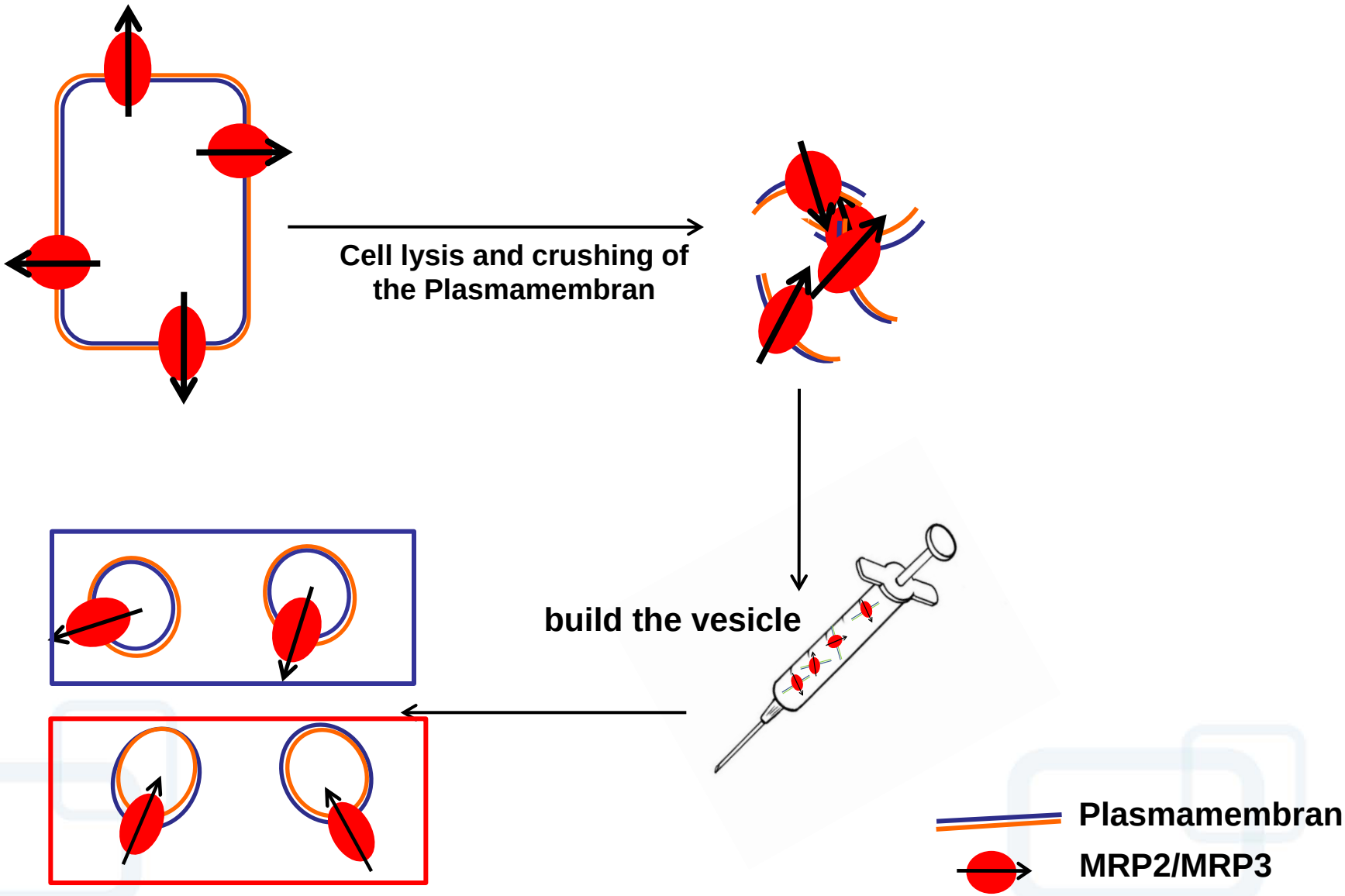
Jia et al., Invest Radiol. 2013； accepted manuscript

Affinity of Gd-EOB-DTPA (Primovist®) to uptake transporters

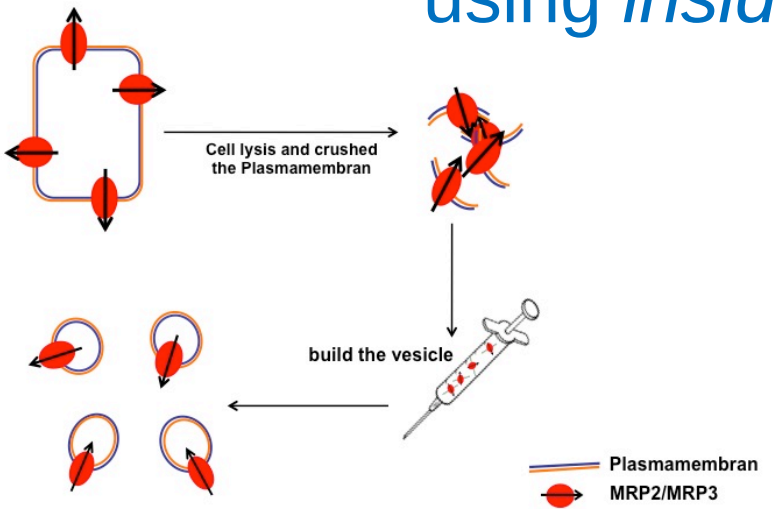
	K_m (mmol/l)	V_{max} (pmol/mg x min)
OATP1B1	1,2	6,3
OATP1B3	0,5	7,4
NTCP	0,04	1,4
OATP2B1	-	-
ASBT	-	-
OCT3	-	-

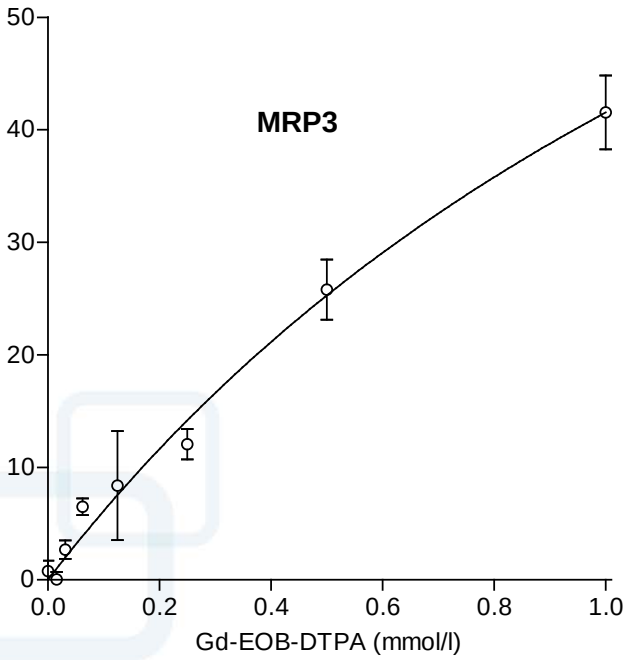
Jia et al., Invest Radiol. 2013； accepted manuscript

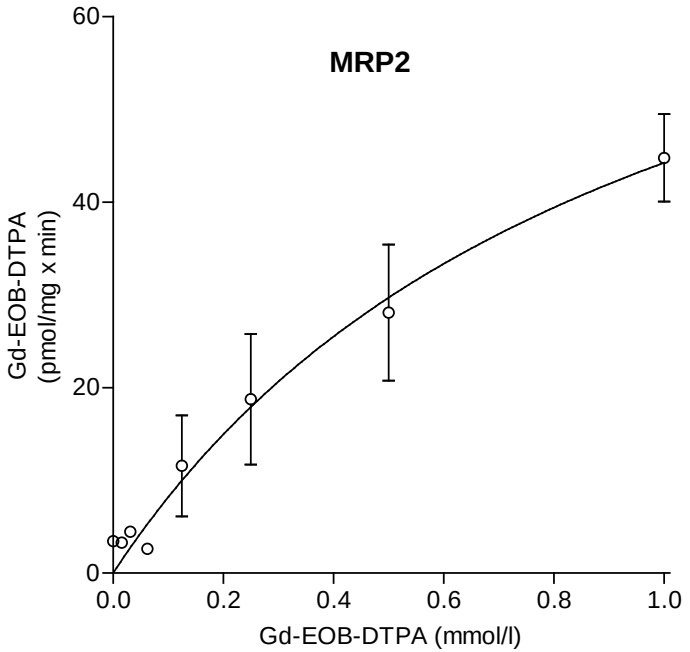
inside-out vesicles



Uptake of Gd-EOB-DTPA (Primovist®) using *inside-out* vesicle







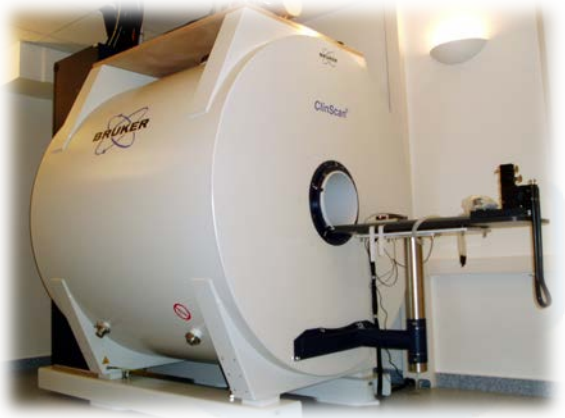
	K_m (mmol/l)	V_{max} (pmol/mg × min)
MRP2	1,0 ± 0,5	86,8 ± 31,3
MRP3	1,8 ± 0,3	116 ± 15,9

Jia et al., Invest Radiol. 2013； accepted manuscript

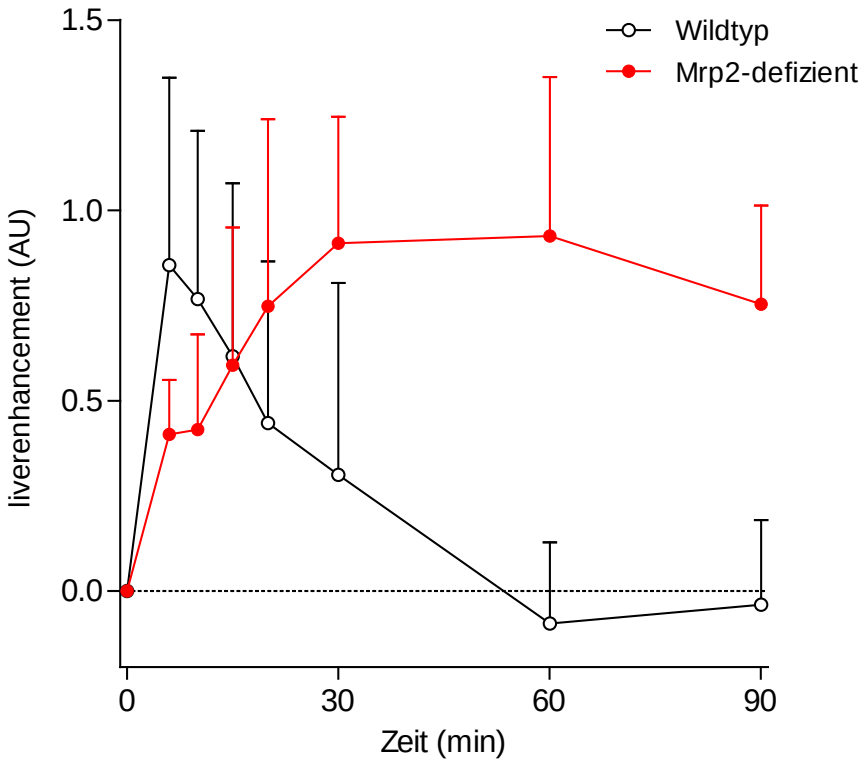
In vivo study

- Animals: wild-type Lewis-rats
  Mrp2-deficient Lewis-rats

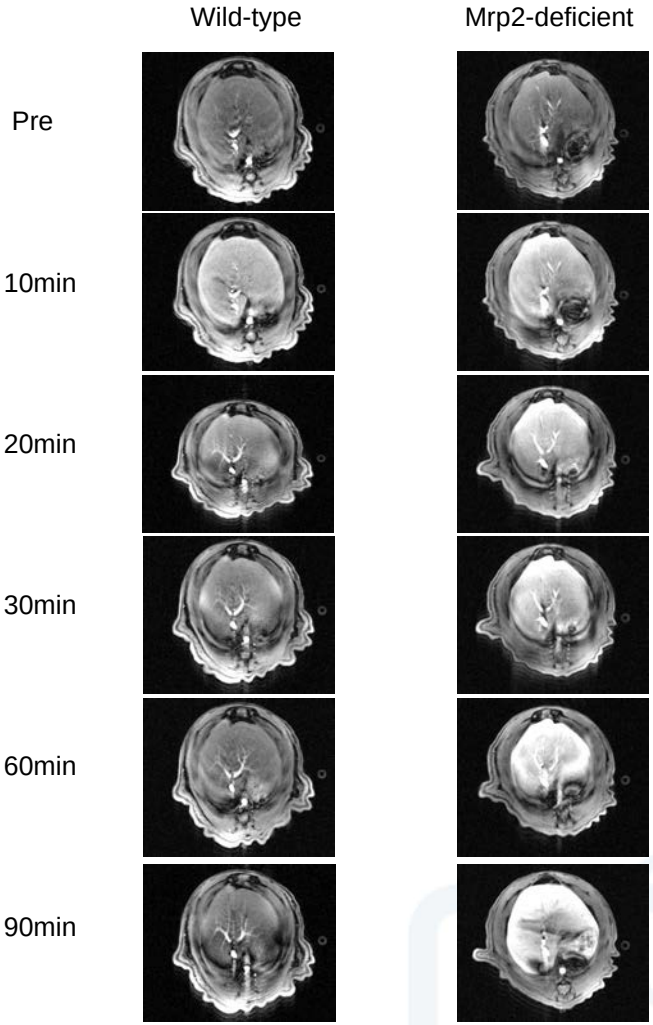
- Operation: Carotis catheter
- MRI: i.v.: bolusinjection 0.025 mmol/kg
  p.o.: 0.025 mmol/kg
- Samples: Blood (i.v: 0-90 min; oral: 0-360min)
  Urine (2d)
  Feces (5d)



MRI: after intravenous application

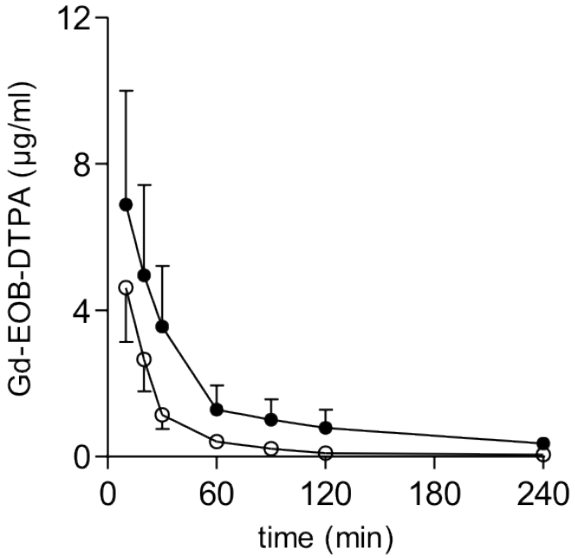


	wild-type	MRP2-deficient
n	8	8
AUC_{0-t} (AU x min)	14.8 ± 10,3	36.4 ± 8.5*
C_{max} (AU)	0.5 ± 0.1	0.5 ± 0.1
T_{max} (min)	6.0 ± 3.1	48.6 ± 23.8*

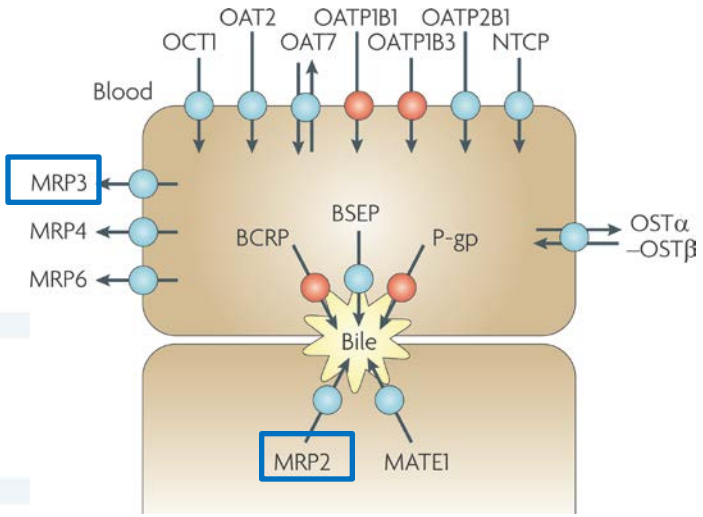


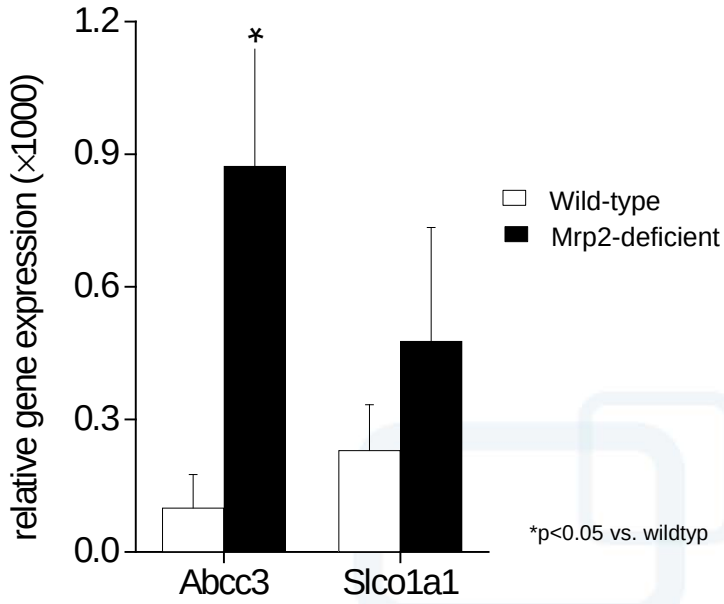
Jia et al., Invest Radiol. 2013； accepted manuscript

Pharmacokinetics: after intravenous applikation



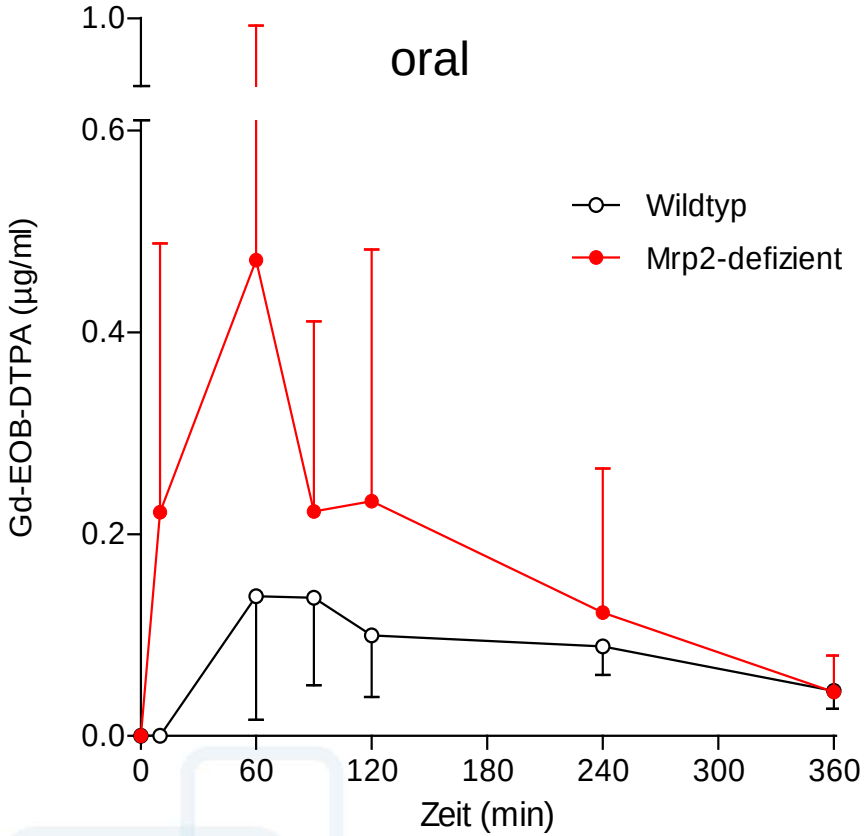
	i.v.	
	wild-type	Mrp2-deficient
AUC $_{0-\infty}$ (µg x h/ml)	3.35	7.49*
C_{p0} (µg/ml)	10	10.4
$T_{1/2}$ (h)	2.12	1.95
$A_{e\ urin}$ (µg)	62.5	666.0
$A_{e\ feces}$ (µg)	1379.0	below LLQ*

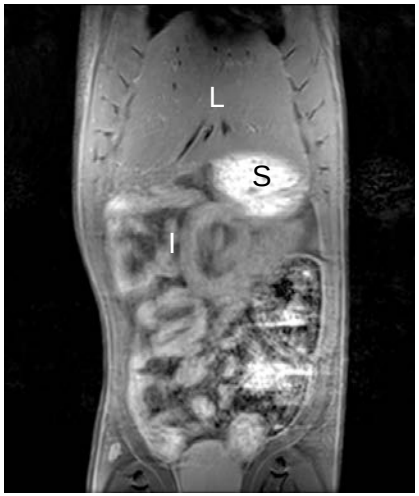




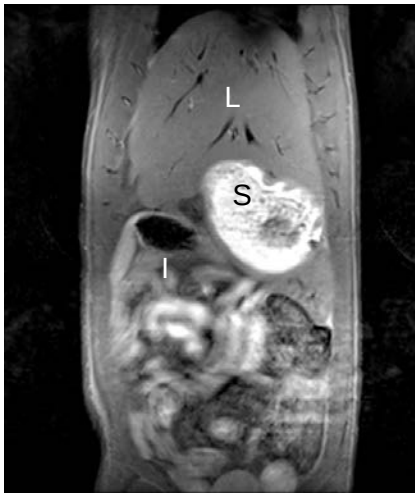
Jia et al., Invest Radiol. 2013; accepted manuscript

Pharmacokinetics and MRI after oral administration





native



nach 40 min

	p.o.	
	Wildtyp	Mrp2-defizient
AUC $_{0-\infty}$ (µg x h/ml)	0,6	1,6
C_{p0} (µg/ml)	0,2	0,5
T_{max} (h)	1,3	0,9
Bioavailability (F)	17%	21%
$A_{e\,urin}$ (µg)	29,7	194,0
$A_{e\,feces}$ (µg)	3511,0	3775,0

Jia et al., Invest Radiol. 2013; accepted manuscript

Conclusion

- The liver-specific uptake of Gd-EOB-DTPA (Primovist®) is realized by OATP1B1 and OATP1B3
- MRP2 is a major efflux transporter of the hepatobiliary elimination
- Cell-based in vitro assays have the potential to replace in vivo animal testing and provide reliable data
- Visualization by MRI can probably replace the quantitative determination of Gd-EOB-DTPA (Primovist®) in liver samples

→ reduced nearly 90% number of experimental animals

Acknowledgment

Institute of pharmacology

Prof. Dr. Werner Siegmund
Prof. Dr. Stefan Oswald
Dr. Markus Keiser
Dr. Jette Penski
Dr. Gabriele Jedlitschky
Dr. Markus Grube
Danilo Wegner
Marten Möller
Gitta Schumacher

Institute of radiology

Prof. Dr. Norbert Hosten
Dr. Dorothee Puls
Dr. Jens-Peter Kühn
Stefan Hadlich

Institute of Pharmacy

Prof. Dr. Werner Weitschies
Dr. Gunnar Glöckl
Dr. Stefan Nagel

Bundesministerium für Bildung und Forschung

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