# EpiIntestinal microtissues:
## An *in vitro* model for the study of first-pass drug metabolism and transport

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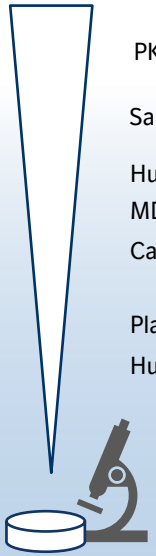
# ADME
## in vitro screening assays reduce number of animal experiments



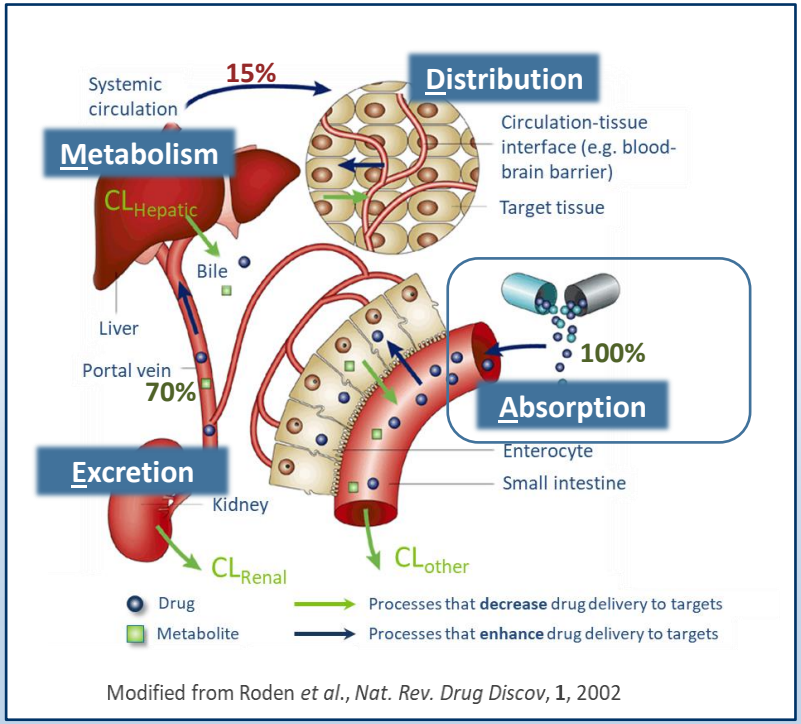


PK studies in animals

Sandwich-cultured human hepatocytes

Human cryo. hepatocytes
MDCK-MDR1 permeability
Caco-2 permeability

Plasma protein binding
Human liver microsomes




Modified from Roden *et al.*, *Nat. Rev. Drug Discov*, **1**, 2002

$$F = F_a \times F_g \times F_h$$

Permeability
Transporter

GI metabolism

Hepatic extraction

# Oral drug absorption: Caco-2 doesn‘t tell the whole story

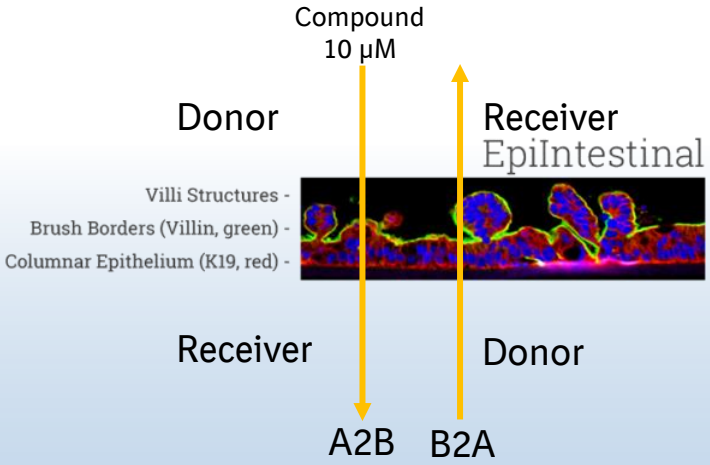
- Advantages of Caco-2 model
  - Immortalized cell line
  - Automated cell culture, automated permeability assay
  - Tight monolayer, relevant P-gp expression level
  - Good in vitro/in vivo correlation regarding fraction absorbed in human intestine

- Disadvantages of Caco-2 model
  - CYP3A4 activities missing
  - Originated from colon, not from small intestine → expression of other drug metabolizing enzymes and transporters may differ

- Additional models in evaluation
  - Human intestinal organoids → Gut-on-chip
  - Static tissue models: e.g. EpiIntestinal

# Prediction of first-pass availability of drugs: Assay setup

## Barrier and transporter function




Readouts:

- Apparent permeability coefficient
- Efflux ratio (sink condition)

# Drug permeability: EpiIntestinal vs. Caco-2

| | Caco-2 | | EpiIntestinal | | Putative transporters |
|---|---|---|---|---|---|
| | PappAB ($10^{-6}$ cm/s) | Efflux | PappAB ($10^{-6}$ cm/s) | Efflux | |
| Fexofenadine | 2.2 | 2.4 | 1.2 | 2.3 | MRP2/Pgp |
| BI 1 | 1.1 | 8.1 | 3.2 | 7.4 | OATP1B1/MRP2 |
| BI 2 | 1.8 | 20.1 | 0.5 | 30.2 | P-gp |
| BI 3 | 0.5 | 1.2 | 2.3 | 1.4 | OCT1 |
| BI 4 | 0.6 | 0.7 | 0.4 | 3.5 | OCT1/Pgp |
| BI 5 | 0.3 | 2.9 | 0.2 | 12.8 | OCT1/Pgp |
| BI 6 | 0.8 | 0.4 | 0.4 | 11.6 | OCT1/Pgp |

- Low permeable compounds tend to show higher efflux in EpiIntestinal compared to Caco-2 → Additional transporter(s) in EpiIntestinal

# Rosuvastatin: Impact of BCRP inhibition on bioavailability

1521-009X/44/3/398–408$25.00
Drug Metabolism and Disposition

http://dx.doi.org/10.1124/dmd.115.066795
Drug Metab Dispos 44:398–408, March 2016

**Solitary Inhibition of the Breast Cancer Resistance Protein Efflux Transporter Results in a Clinically Significant Drug-Drug Interaction with Rosuvastatin by Causing up to a 2-Fold Increase in Statin Exposure**

Robert Elsby,[1] Paul Martin, Dominic Surry,[2] Pradeep Sharma, and Katherine Fenner

| | | EpiIntestinal | | | Caco-2 | | |
|---|---|---|---|---|---|---|---|
| Substrate | Inhibitor | AB (e-6 cm/s) | BA (e-6 cm/s) | Efflux | AB (e-6 cm/s) | BA (e-6 cm/s) | Efflux |
| Rosuvastatin | None | 0.3 | 29 | 100 | 0.3 | 6.8 | 21 |
| | Lapatinib | 2.0 | 7.7 | 3.9 | 0.6 | 1.3 | 2.2 |
| | Ko143 | 2.6 | 7.9 | 3.1 | 0.5 | 2.9 | 5.5 |
| | Zosuquidar | 0.9 | 21 | 25 | 0.3 | 4.6 | 19 |

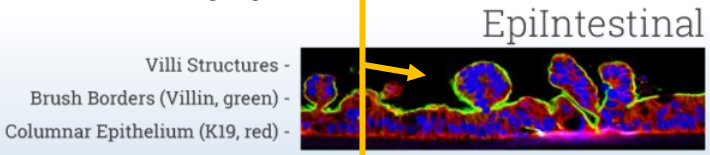
- Both in EpiIntestinal model and Caco-2 cellular model, BCRP is the major factor limiting Rosuvastatin absorption → Consistent with literature data

# Prediction of first-pass availability of drugs: Assay setup

## Firstpass availability

Compound
10 µM

Donor

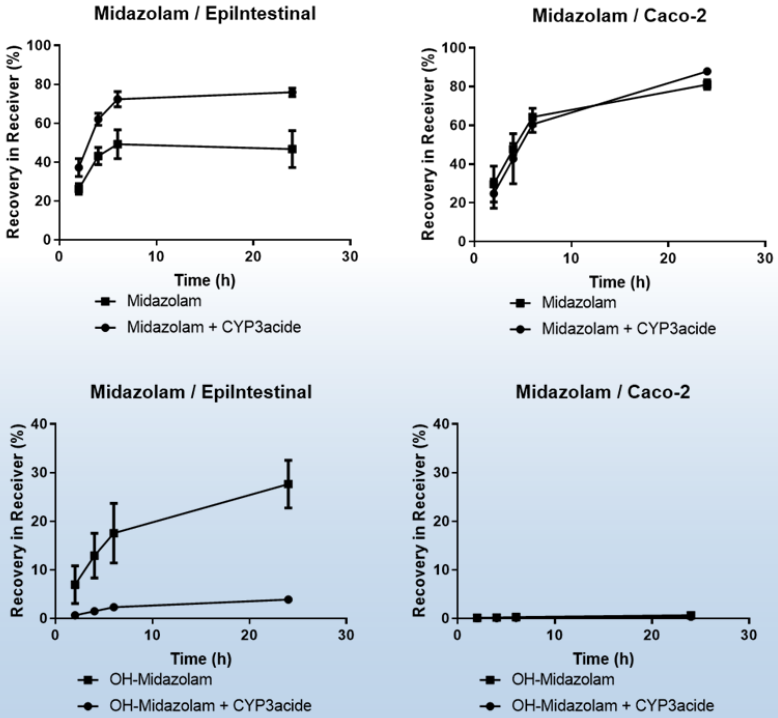



Receiver

Readouts:

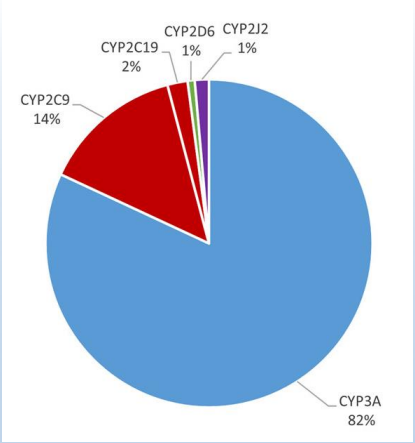
- Time-dependent recovery of parent in Donor and Receiver
- Recovery in lysate at the end of experiment
- Efflux ratio (under steady state)
- Donor and receiver samples stored for MetID

# Intestinal CYP3A activities: EpiIntestinal vs. Caco-2

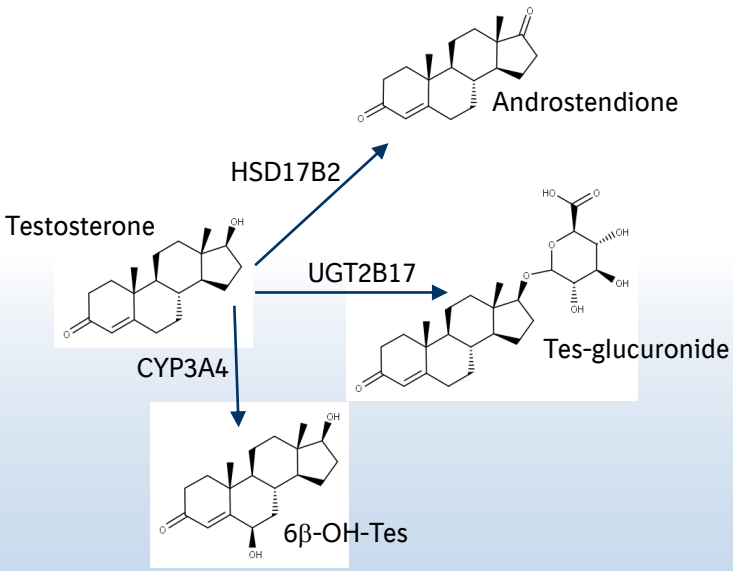


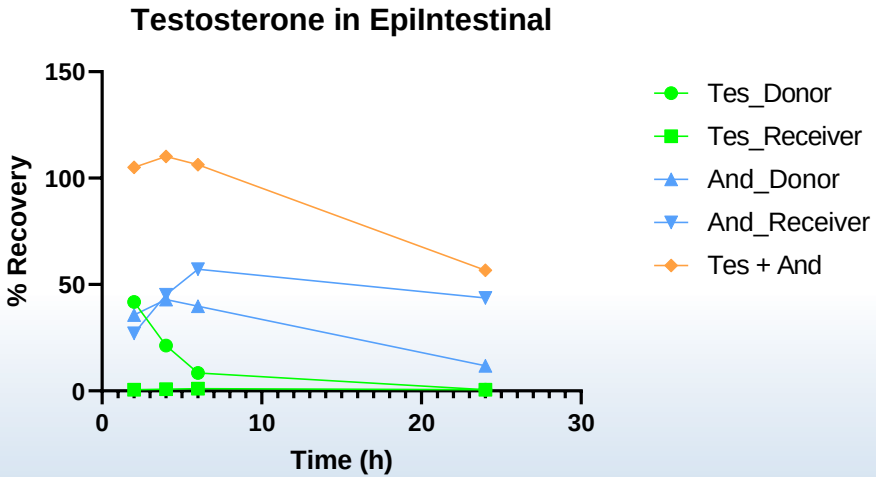

- CYP3A activities limit the receiver (blood) availability of midazolam in EpiIntestinal
- CYP3A activities absent in Caco-2

Human intestine



# Testosterone metabolism: Hepatocyte vs. Enterocytes vs. EpiIntestinal




| | Hepatocyte (% of Met)* | Enterocyte (% of Met)* | EpiIntestinal (% of Met) |
|---|---|---|---|
| Androstendione | 60 | 89 | 98 |
| Tes-glucuronide | 20 | 7 | 2 |
| 6β-OH-Tes | 11 | 3 | Trace |

*: Taken from Zhang et al., Biochem Pharmacol, 156:32 (2018)

→ Low recovery of Tes in Receiver of EpiIntestinal fits to the low oral bioavailability in human
→ Metabolite pattern in EpiIntestinal close to human enterocytes

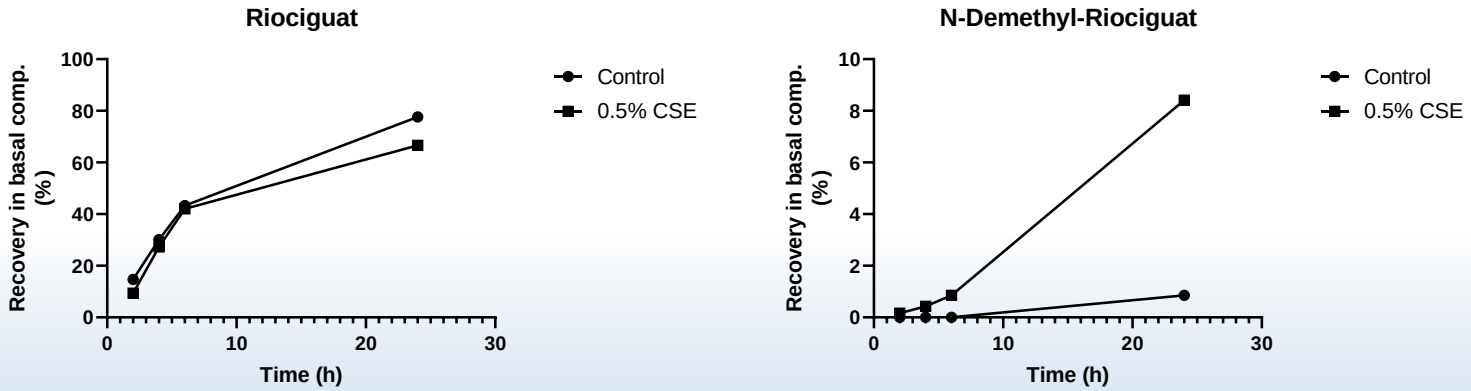
# Glucuronidation: EpiIntestinal vs. Isolated human intestinal mucosa

| | EpiIntestinal | | Human intestinal mucosa | |
|---|---|---|---|---|
| | Ezetimibe | Raloxifene | Ezetimibe | Raloxifene |
| Remaining parent after incubation (% of T0) | 8.4 | 2.4 | 40.3 | 33.2 |
| Metabolites (% of PA of parent drug @ T0) | | | | |
| Glucuronides | 39.1 | 2.2 | 58.6 | 18.7 |
| Sulfate | | 14.1 | | 2.0 |

→ UGT and SULT activities identified in EpiIntestial

→ Similar metabolite pattern in EpiIntestinal and isolated human intestinal mucosa

# EpiIntestinal: Induction of CYP1A1 by cigarette smoke extract




- Strong increase of CYP1A1 metabolite upon treatment with CSE
- Minor effect of CSE on availability of Riociguat in receiver (consistent with clinic observation)

# EpiIntestinal vs Caco-2: Basal enzyme activity screen

| | Caco-2 | | EpiIntestinal | |
|---|---|---|---|---|
| DME/Substrate | Enzyme activities* (pmol/h/cm2) mean / SD | Intracellular metabolite (% of total) | Enzyme activities* (pmol/h/cm2) mean / SD | Intracellular metabolite (% of total) |
| CYP1A2/Phenacetin | 123.1 / 4.8 | BLQ | 17.4 / 3.0 | BLQ |
| CYP2B6/Bupropion | BLQ | BLQ | 2.6 / 0.9 | BLQ |
| CYP2C8/Amodiaquine | 11.2 / 1.9 | 36.5 | 107.9 / 49.1 | 37.5 |
| CYP2C9/Diclofenac | 20.8 / 1.6 | 12.8 | 28.4 / 1.6 | 14.7 |
| CYP2C19/S-Mephenytoin | 7.1 / 0.7 | 4.5 | 6.9 / 0.7 | 4.3 |
| CYP3A4/Testosterone | 26.5 / 3.6 | BLQ | 176.4 /8.0 | 0.9 |
| CYP3A4/Midazolam | BLQ | BLQ | 1.9 / 0.5 | 14.9 |
| CYP2D6/Dextromethorphan | 10.9 / 2.7 | BLQ | 9.2 / 1.3 | BLQ |
| UGT/7-OH-Coumarin | 10770.5 / 721.9 | 5.5 | 7583.4 / 855.2 | 10.0 |
| SULT/7-OH-Coumarin | 508.0 / 46.1 | BLQ | 1747.4 / 140.0 | 3.4 |
| UGT1A1/β-Estradiol | 65.3 / 6.9 | 3.2 | 243.3 / 6.7 | 2.8 |
| CYP2J2/Astemizole | 4.9 / 0.3 | 62.9 | 17.7 / 5.2 | 68.7 |
| CES/BIBF 1120 | 370.9 / 31.4 | 24.7 | 400.0 /12.9 | 13.3 |

- Major differences in drug-metabolizing enzymes

# Prediction of human Fa*Fg using EpiIntestinal model

| | Recovery (%) basal comp. @24h | Fa*Fg (%) | F (%) | DME/transporter |
|---|---|---|---|---|
| Buspirone | **60** | **70** | 5 | CYP3A |
| Indinavir | **53** | **100** | 60 | CYP3A |
| Saquinavir | **18** | **25** | 4 | CYP3A |
| Atorvastatin | **43** | **28** | 14 | CYP3A/BCRP/MRP2 |
| Rosuvastatin | **30** | **62** | 20 | CYP2C9/BCRP/MRP2 |
| Felodipine | **47** | **62** | 15 | CYP3A |
| Nifedipine | **110** | **100** | 90 | CYP3A |
| Quinidine | **85** | **100** | 90 | CYP3A4, etc. |
| Oxybutynin | **16** | **9** | 6 | Esterase, CYP3A |
| Midazolam | **47** | **67** | 40 | CYP3A4 |
| Irinotecan | **62** | **39** | 25 | Esterase, CYP3A4 |
| Atenolol | **83** | **50** | 50 | |
| Raloxifene | **25** | **5** | 2 | UGT/Sult |
| Astemizole | **13** | | **Low** | CYP2J2, etc. |
| Bazedoxifene | **27** | | **6** | UGT |

- Less than 2-fold difference to human data for all compounds tested
- Compounds with extensive intestinal metabolism well captured by EpiIntestinal

# Summary

- Most of the relevant DME and drug transporters identified in EpiIntestinal
- Clear advantage of EpiIntestinal compared to Caco-2:
  - CYP3A activities completely absent in Caco-2
  - Higher activities of major DMEs in EpiIntestal than in Caco-2

→EpiIntestinal is a useful tool
- For the prediction of human $F_a$*$F_g$ (→Solubility to be considered separately)
- For mechanistic understanding of DDI in GI tract
- For identification of extrahepatic metabolic pathways

- Future perspective
  - Availability of similar models for preclinical animal species
  - Combination with microfluidics: Any additional benefits?