14th European ISSX Meeting, Gürzenich, Köln, Germany
26 June 2017

Pharmacokinetic Evaluation of Transporter Probe Drugs *in vivo* and their Combination in a Cocktail

Fabian Müller

Translational Medicine and Clinical Pharmacology,
Boehringer Ingelheim Pharma GmbH & Co. KG

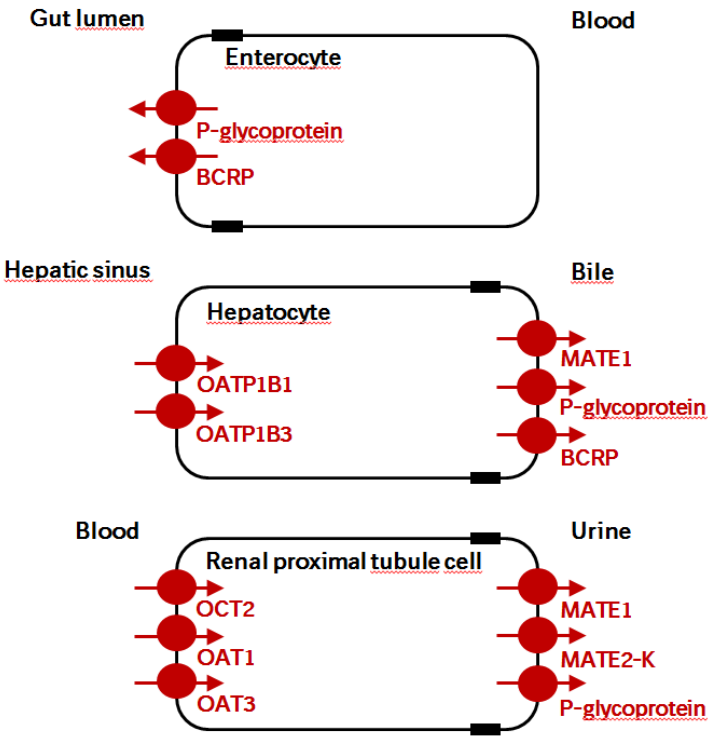
Core Project Team: Peter Stopfer, Thomas Gießmann, Fabian Müller, Kathrin Hohl, Naoki Ishiguro, Ashish Sharma, Heike Zimdahl-Gelling, Mitchell E. Taub and Thomas Ebner

Boehringer Ingelheim

Transporter-based drug-drug interactions

- Inhibition (or induction) of drug transporters may cause drug-drug interactions (DDIs).
- The effect of a new molecular entity on relevant drug transporters needs to be assessed during drug development.
- The potential of a new molecular entity for inhibition of drug transporters is usually assessed by means of *in vitro* experiments.
- *In vitro* inhibition data are compared with guideline-derived cut-off values for decision on further investigation in clinical trials.

Select drug transporters in the intestine, liver and kidney:



- Giacomini KM et al. Nat Rev Drug Discov. 2010 9:215-36.
- Hillgren KM et al. Clin Pharmacol Ther. 2013 94:52-63.
- EMA Guideline on the investigation of drug interactions; 21 June 2012 CPMP/EWP/560/95/Rev. 1 Corr. 2
- König J et al. Pharmacol Rev. 2013 65:944-66.

Investigation of transporter-based DDIs *in vivo*

- For a new molecular entity, several clinical trials may become necessary to investigate transporter-based DDIs with the new molecular entity as a potential perpetrator drug.

- An example from medical literature:

  The effect of isavuconazole on probe drugs for drug transporters was investigated in four separate clinical trials in healthy volunteers. These trials investigated the effect of isavuconazole on plasma exposure of the following probe drugs:

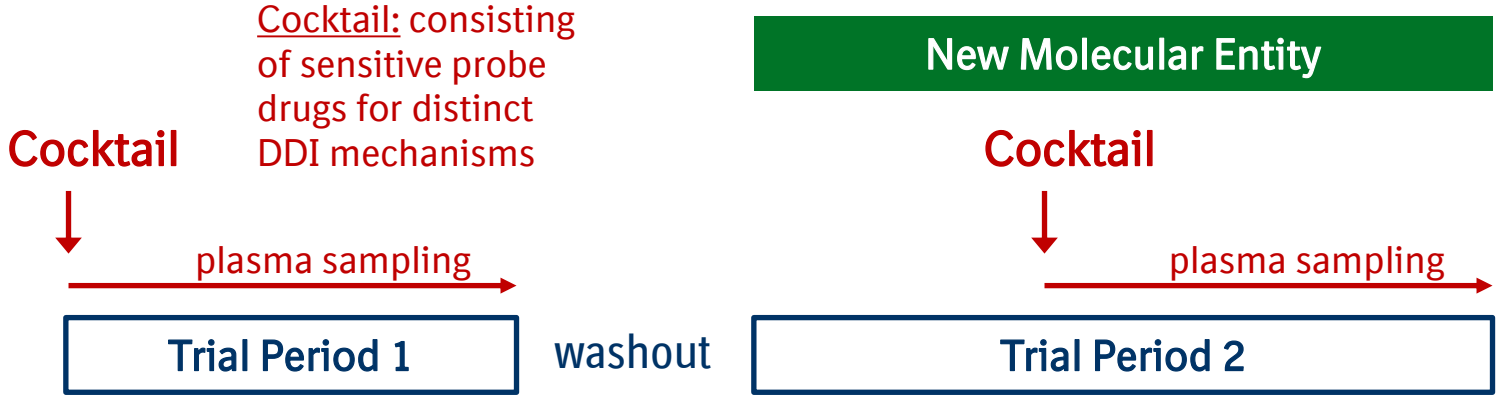
  - Trial A: **Atorvastatin** (target transporters: OATP1B1, P-glycoprotein)
  - Trial B: **Digoxin** (target transporter: P-glycoprotein)
  - Trial C: **Metformin** (target transporters: OCT1, OCT2, MATE1)
  - Trial D: **Methotrexate** (target transporters: BCRP, OAT1, OAT3)

Yamazaki T et al. Clin Pharmacol Drug Dev. 2017 6:66-75.

The cocktail approach: Overview

A probe drug cocktail approach may be applied to simultaneously assess several distinct DDI mechanisms with a new molecular entity as potential perpetrator drug within one single trial.

A possible trial design:



⇒ Reduction of the number of DDI studies in a development program

⇒ Budget and resource savings

⇒ Reduced number of subjects exposed to new molecular entity

The cocktail approach: The regulatory perspective - EMA

EMA:

- ‘It is possible to use so called “cocktail studies” to investigate the effects of an investigational drug on several enzymes and transporters in one *in vivo* study.’
- ‘*In vivo* cocktail studies may also be used to replace studies of the *in vitro* inhibition and induction potential of parent drug (and metabolites) on enzymes (and transporters).’
- ‘If satisfactorily performed, the results of the cocktail studies can be extrapolated to other drugs and can be used to support treatment recommendations in the SmPC.’
- ‘It should have been demonstrated *in vivo* that the probe drugs combined in the “cocktail” do not interact with each other.’

EMA Guideline on the investigation of drug interactions; 21 June 2012 CPMP/EWP/560/95/Rev. 1 Corr. 2

The cocktail approach: The regulatory perspective - FDA

FDA:

- ‘Negative results from a well-conducted cocktail study may eliminate the need for further evaluation of particular CYP enzymes and transporters. However, positive results may indicate that further in vivo evaluation should be conducted.’
- ‘The data generated from a cocktail study can supplement data from other in vitro and in vivo studies in assessing a drug’s potential to inhibit or induce CYP enzymes and transporters.’
- Requirements:
  - ‘(1) the substrates are specific for individual CYP enzymes or transporters’
  - ‘(2) there are no interactions among these substrates’
  - ‘(3) the study is conducted in a sufficient number of subjects’

FDA Guidance for Industry: Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations; Draft Guidance; February 2012.

The Boehringer Ingelheim Transporter Cocktail Project

Literature search | ***In vitro* evaluation** | **External experts**

Cocktail Probe Drugs *

- **Digoxin** (P-glycoprotein)
- **Furosemide** (OAT1, OAT3)
- **Metformin** (OCT2, MATE1, MATE2-K)
- **Rosuvastatin** (OATP1B1, OATP1B3, BCRP)

- ✓ **Metabolism:** minimal / negligible
- ✓ **Transport:** *in vitro* evidence & *in vivo* sensitivity to inhibition
- ✓ **Safety:** suited for use in DDI studies
- ✓ **Mutual DDIs:** no evidence for relevant mutual interaction
- ✓ **Availability:** worldwide

***In vivo* evaluation (Clinical Trials)**

Step 1: Exclusion of mutual DDIs - 3 trials

Step 2: Test of sensitivity

* Ebner T et al. J Pharm Sci. 2015 104:3220-8

Step 1 / Trial 1 – Aim, Design, Treatments

Aim:
- Investigation of the relative bioavailability of the four cocktail compounds given alone compared to when given in combination as cocktail
- Investigation of the effect of higher doses of metformin or furosemide on pharmacokinetics of the other cocktail compounds

Design: Open-label, randomised, six-period crossover trial in 24 healthy male volunteers

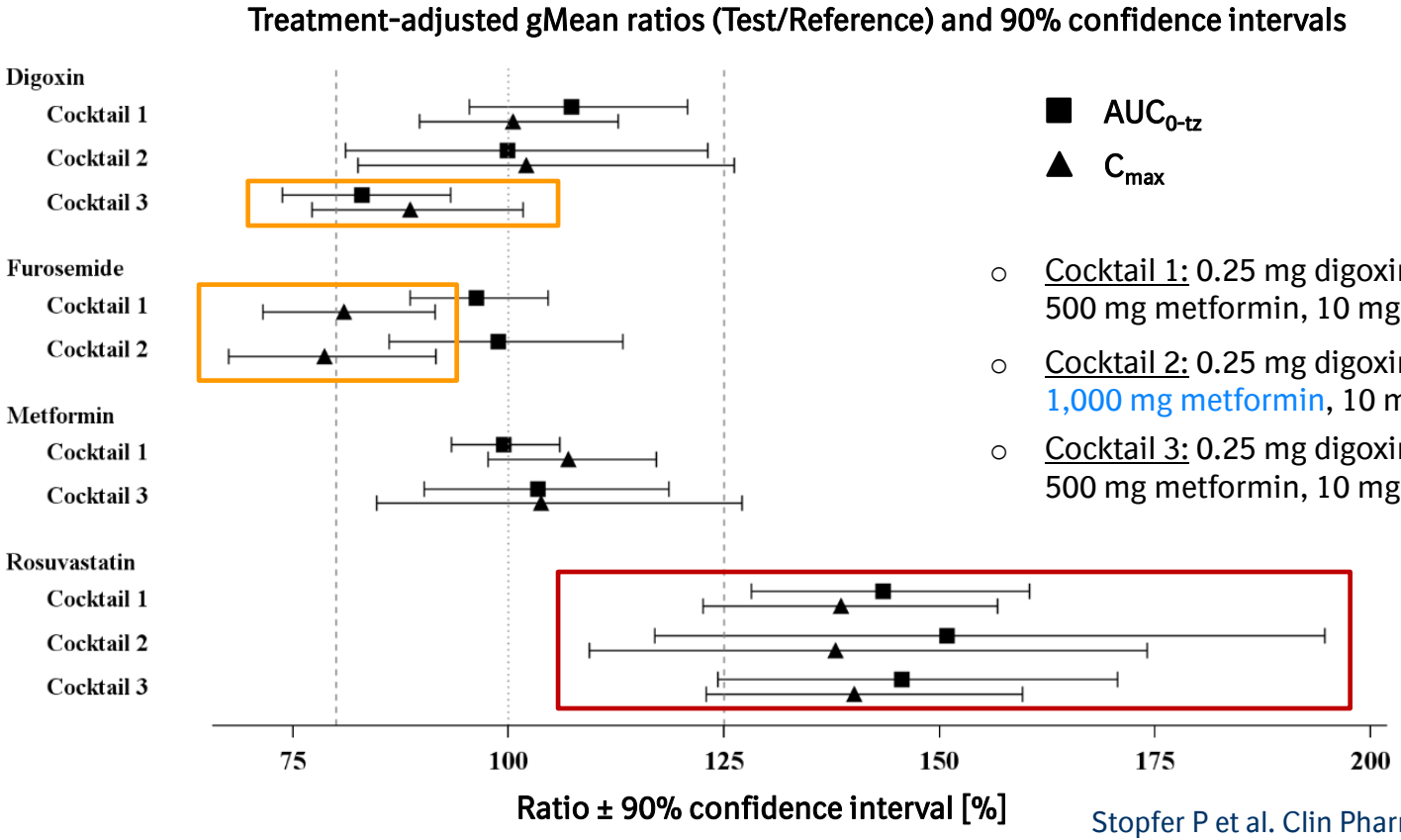
Treatments:

Reference 1	(N=22) Mono-treatment: **digoxin 0.25 mg**
Reference 2	(N=22) Mono-treatment: **furosemide 5 mg**
Reference 3	(N=22) Mono-treatment: **metformin 500 mg**
Reference 4	(N=22) Mono-treatment: **rosuvastatin 10 mg**
Test 1	(N=22) **Cocktail:** all four drugs together
Test 2	(N=11) Cocktail: containing a 2x metformin dose (1,000 mg)
Test 3	(N=12) Cocktail: containing a 4x furosemide dose (20 mg)

N: number analysed for primary endpoint

Stopfer P et al. Clin Pharmacol Ther. 2016 100:259-67

Step 1 / Trial 1 – Results



■ AUC_{0-tz}

▲ C_{max}

- Cocktail 1: 0.25 mg digoxin, 5 mg furosemide, 500 mg metformin, 10 mg rosuvastatin
- Cocktail 2: 0.25 mg digoxin, 5 mg furosemide, 1,000 mg metformin, 10 mg rosuvastatin
- Cocktail 3: 0.25 mg digoxin, 20 mg furosemide, 500 mg metformin, 10 mg rosuvastatin

Stopfer P et al. Clin Pharmacol Ther. 2016 100:259-67

Step 1 / Trial 2 – Aim, Design, Treatments

Aim: Investigation of the relative bioavailability of rosuvastatin when given alone compared to when given together with different doses of metformin or furosemide

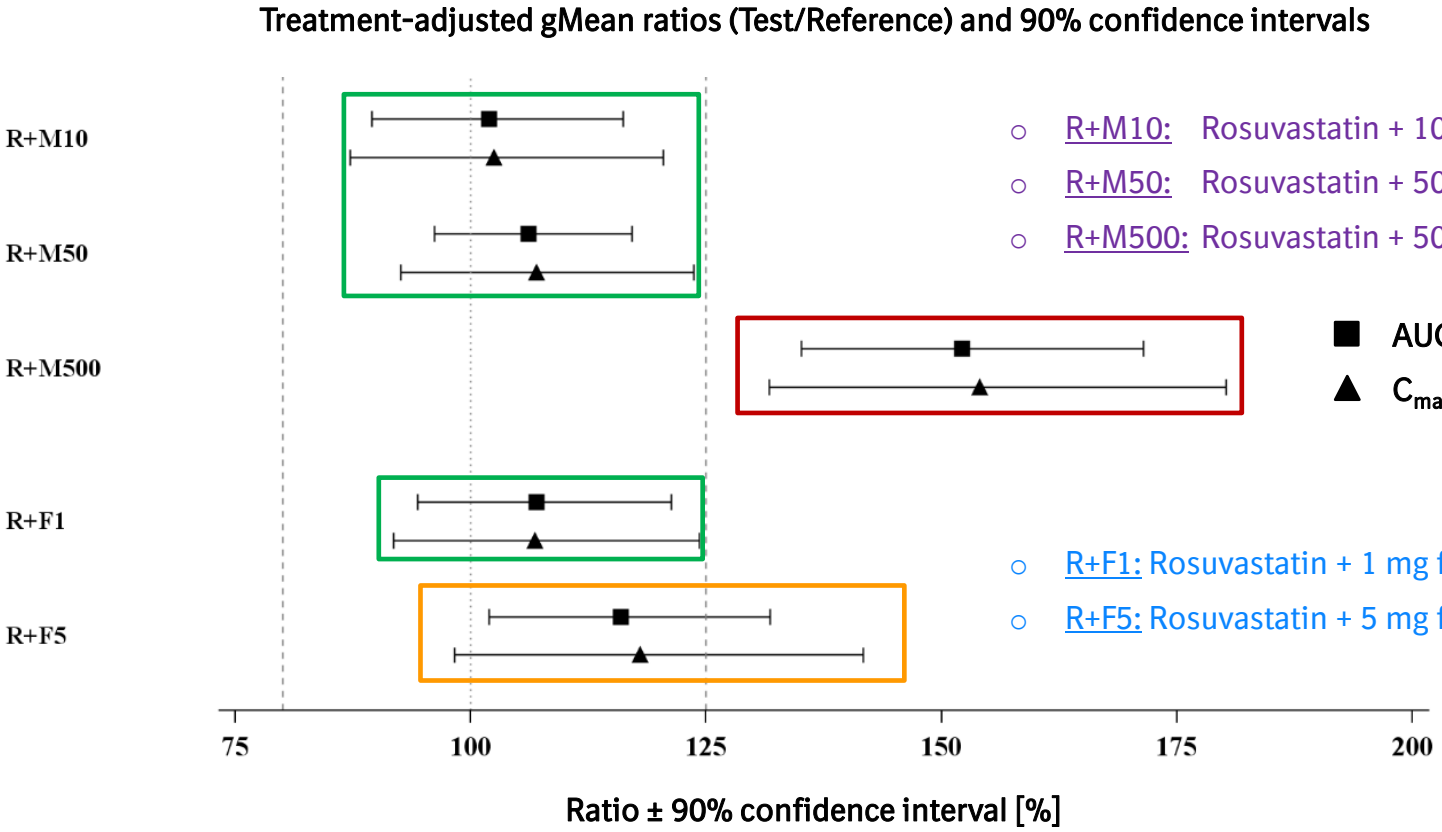
Design: Open-label, randomised, six-period crossover trial in 18 healthy male volunteers

Treatments:

Reference	(N=16)	Rosuvastatin 10 mg **alone**
Test 1	(N=15)	Rosuvastatin 10 mg **+ 10 mg metformin**
Test 2	(N=16)	Rosuvastatin 10 mg **+ 50 mg metformin**
Test 3	(N=15)	Rosuvastatin 10 mg **+ 500 mg metformin**
Test 4	(N=15)	Rosuvastatin 10 mg **+ 1 mg furosemide**
Test 5	(N=16)	Rosuvastatin 10 mg **+ 5 mg furosemide**

N: number analysed for primary endpoint

Step 1 / Trial 2 – Results



- R+M10: Rosuvastatin + 10 mg metformin
- R+M50: Rosuvastatin + 50 mg metformin
- R+M500: Rosuvastatin + 500 mg metformin

- R+F1: Rosuvastatin + 1 mg furosemide
- R+F5: Rosuvastatin + 5 mg furosemide

Step 1 / Trial 3 – Aim, Design, Treatments

Aim: Investigation of mutual pharmacokinetic interactions of the four probe drugs given together in an optimized probe drug cocktail containing reduced doses of metformin (10 mg) and furosemide (1 mg).

Design: Open-label, randomised, five-period crossover trial in 30 healthy male volunteers

Treatments:

Reference 1 (N=28) Mono-treatment: **digoxin 0.25 mg**
Reference 2 (N=30) Mono-treatment: **furosemide 1 mg** *(instead of 5 mg in the first cocktail)*
Reference 3 (N=29) Mono-treatment: **metformin 10 mg** *(instead of 500 mg in the first cocktail)*
Reference 4 (N=29) Mono-treatment: **rosuvastatin 10 mg**

Test (N=28) **Cocktail:** all four drugs together

N: number analysed for primary endpoint

Step 1 / Trial 3 – Results

Endpoint	Ratio T/R[1] [%]	90% CI [%]	gCV[2] [%]	Endpoint	Ratio T/R[1] [%]	90% CI [%]	gCV[2] [%]
Digoxin				**Metformin**			
AUC_{0-tz}	**96.39**	(88.22; 105.33)	19.4	AUC_{0-tz}	**97.49**	(93.54; 101.61)	9.0
C_{max}	**93.17**	(83.49; 103.97)	24.1	C_{max}	**98.25**	(91.85; 105.09)	14.7
$AUC_{0-\infty}$	**96.53**	(92.08; 101.20)	10.1	$AUC_{0-\infty}$	**97.50**	(93.58; 101.58)	8.9
Furosemide				**Rosuvastatin**			
AUC_{0-tz}	**102.62**	(93.82; 112.25)	20.4	AUC_{0-tz}	**105.01**	(96.39; 114.40)	18.8
C_{max}	**103.96**	(93.60; 115.46)	24.0	C_{max}	**104.28**	(94.95; 114.53)	20.6
$AUC_{0-\infty}$	**97.40**	(90.87; 104.41)	9.6	$AUC_{0-\infty}$	**107.63**	(97.04; 119.39)	19.4

[1] Treatment-adjusted gMean ratios (probe drug as cocktail component) / (probe drug mono-treatment)

[2] Intraindividual

Step 1 / Trial 3 – Results

Endpoint	Ratio T/R[1] [%]	90% CI [%]	gCV[2] [%]	Endpoint	Ratio T/R[1] [%]	90% CI [%]	gCV[2] [%]
Digoxin				**Metformin**			
fe_{0-36}	**99.43**	(93.93; 105.26)	12.2	fe_{0-24}	**98.57**	(93.24, 104.21)	12.0
$CL_{R,0-36}$	**103.03**	(100.18; 105.96)	5.9	$CL_{R,0-24}$	**101.14**	(97.42, 105.00)	8.0
Furosemide				**Rosuvastatin**			
fe_{0-24}	**99.65**	(85.41, 116.28)	35.4	fe_{0-36}	**103.90**	(95.86, 112.62)	16.8
$CL_{R,0-24}$	**91.44**	(77.02, 108.56)	27.6	$CL_{R,0-36}$	**97.52**	(93.20, 102.03)	9.4

[1] Treatment-adjusted gMean ratios (probe drug as cocktail component) / (probe drug mono-treatment)
[2] Intraindividual

Summary and next steps

- Step 1: The transporter probe-drug cocktail consisting of 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin and 10 mg rosuvastatin is free of mutual pharmacokinetic DDI.
  - ✓ Relative bioavailability of digoxin, furosemide, metformin, and rosuvastatin as part of the test cocktail compared to when given alone was similar for $AUC_{0\text{-}tz}$, $AUC_{0\text{-}\infty}$, and C_{max}.
  - ✓ All 90% confidence intervals for adjusted gMean ratios ($AUC_{0\text{-}tz}$, $AUC_{0\text{-}\infty}$, and C_{max}) were within standard bioequivalence acceptance range.
- Step 2: Further clinical validation of this probe drug cocktail is planned with dedicated inhibitors of drug transporters.

Acknowledgments

Core team and sponsors:

- Thomas Ebner
- Thomas Gießmann
- James Hilbert
- Naoki Ishiguro
- Fabian Müller
- Ashish Sharma
- Kathrin Hohl
- Mitchell E. Taub
- Peter Stopfer
- Heike Zimdahl-Gelling
- Mark Castles
- Ulrich Roth
- Florian Gantner

Internal contributors:

- Ayano Fukuhara
- Caroline MacLean
- Christian Lechner
- Meeghan O'Connor
- Naoko Ohtsu
- Lalitha Podila
- Asami Saito
- Hidetada Shimizu
- Junichi Takano
- Masahito Takatani
- Tokuko Takatsuka
- Ikumi Washio
- Walter Weber
- Dietmar Ganßer
- Sven Schmidt
- Ulrike Maikler
- Gerhard Ries
- Sabine Hutzel
- Arvid Jungnik
- Sven Schmidt

External contributors:

- Uwe Fuhr
- Martin Fromm
- Kathleen Giacomini
- Richard Kim
- Mikko Niemi
- Peter Swaan
- Gerd Mikus

