Effects of cytochrome P450 inhibition and induction on the phenotyping metrics of the Basel cocktail: a randomized cross-over study

SUPPLEMENTARY MATERIAL

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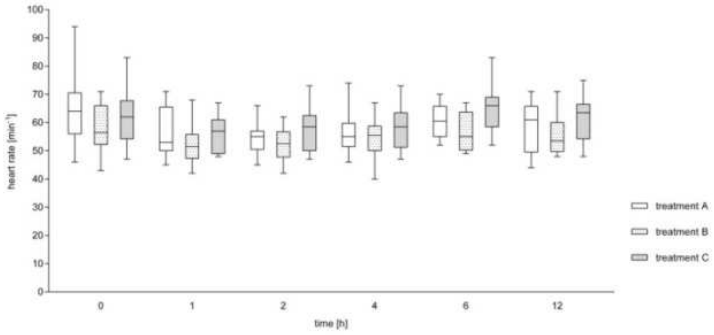
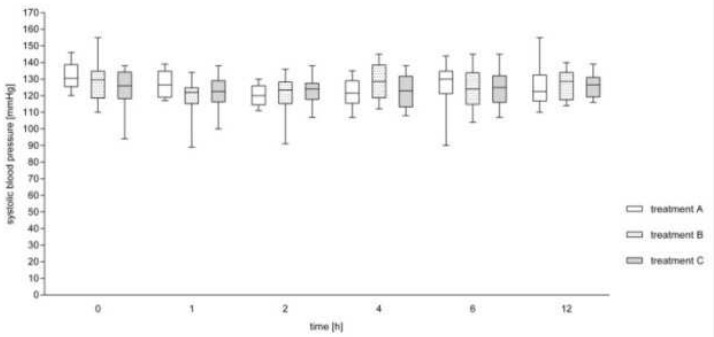
Supplementary Figure 1

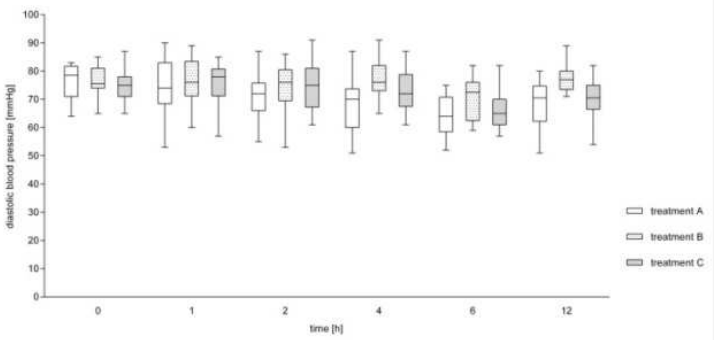
A Heart rate



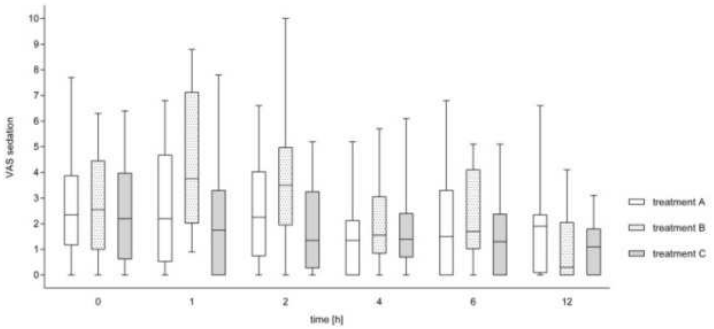
B Systolic blood pressure



C Diastolic blood pressure

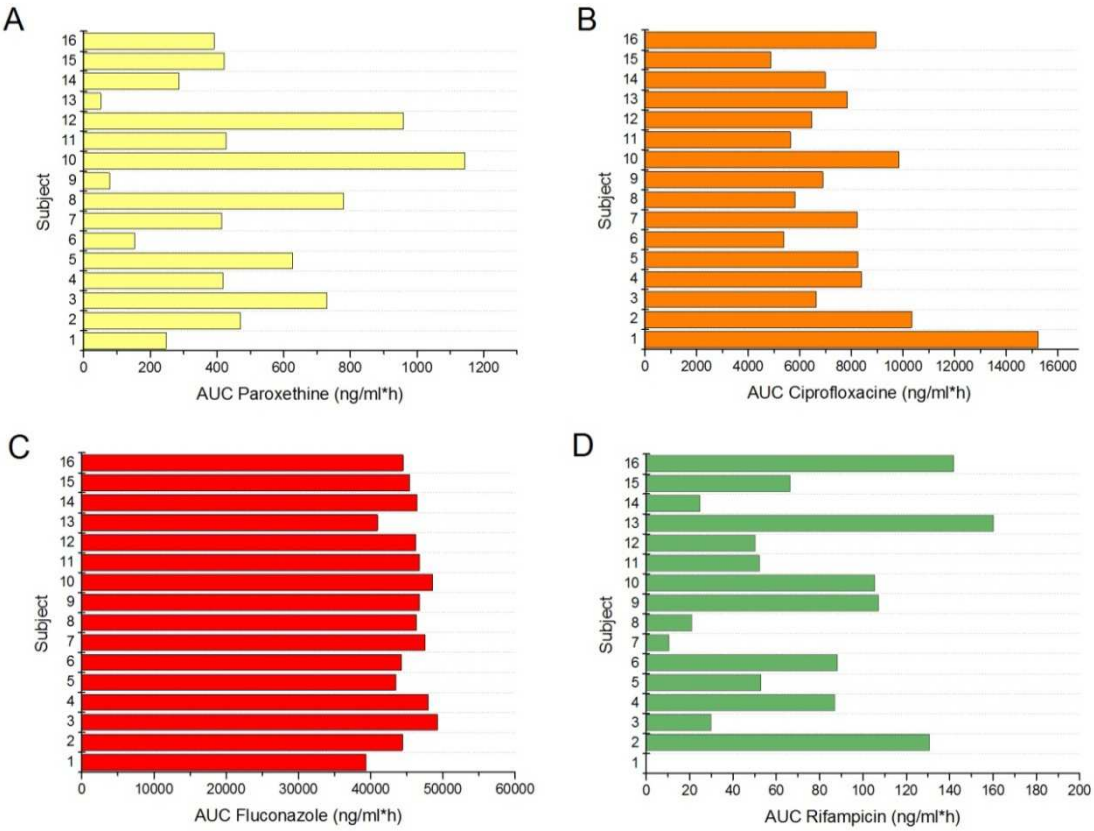


D Sedation score



Heart rate (A), systolic (B) and diastolic (C) blood pressure and sedation scores (D) after administration of the Basel cocktail (baseline/treatment A, white boxes), after pretreatment with the CYP inhibitors ciprofloxacin, paroxetine and ketoconazole (inhibition/treatment B, dotted boxes) and after pretreatment with rifampicin (induction/treatment C, grey boxes). Whiskers represent the range with minimum and maximum, boxes represent the 25.-75. percentiles and the median.

Supplementary Figure 2



Exposure ($AUC_{0\text{-}24h}$) to paroxetine (A), ciprofloxacin (B), fluconazole (C), and rifampicin (D). The last CYP inhibitor dose was taken 1 hour and the last rifampicin dose was taken 24 hours before application of the Basel cocktail. Subject 1 had approx. 100-fold higher rifampicin exposure compared to the other subjects, indicating insufficient auto-induction and was excluded from further analysis of induction data.

Supplementary Table 1: Accuracy and Precision

nominal concentrations		QC1 = 5 ng/mL	QC2 = 50 ng/mL	QC3 = 500 ng/mL
Metoprolol	Overall Mean (n)	5.51 (9)	50.9 (9)	454 (9)
	Accuracy (Bias%)	10.2	1.74	-9.11
	Precision (CV %)	3.55	3.19	3.70
OH-metoprolol	Overall Mean (n)	5.36(8)	49.9 (9)	488 (9)
	Accuracy (Bias%)	7.10	-0.76	-2.36
	Precision (CV %)	8.49	8.67	5.42
Omeprazole	Overall Mean (n)	5.06 (9)	50.6 (9)	457 (9)
	Accuracy (Bias%)	1.36	1.020	-8.62
	Precision (CV %)	4.84	3.15	2.48
5-OH-omeprazole	Overall Mean (n)	5.05 (9)	50.3 (9)	442 (9)
	Accuracy (Bias%)	0.860	0.670	-11.6
	Precision (CV %)	5.20	5.33	3.28
Losartan	Overall Mean (n)	5.24 (9)	51.4 (9)	not measured
	Accuracy (Bias%)	4.80	6.68	
	Precision (CV %)	8.10	6.68	
E-3174	Overall Mean (n)	5.28 (9)	52.4 (9)	460 (9)
	Accuracy (Bias%)	5.57	4.76	-8.04
	Precision (CV %)	8.24	5.70	4.58
Midazolam	Overall Mean (n)	5.41 (6)	49.9 (9)	not measured
	Accuracy (Bias%)	8.12	-0.18	
	Precision (CV %)	6.20	6.98	
1'-OH-midazolam	Overall Mean (n)	5.11 (8)	49.3 (8)	486 (9)
	Accuracy (Bias%)	2.16	-1.49	-2.90
	Precision (CV %)	9.11	4.29	2.64
Caffeine	Overall Mean (n)	not measured	50.8	506
	Accuracy (Bias%)		1.64	1.28
	Precision (CV %)		10.40	6.22
Paraxanthine	Overall Mean (n)	not measured	50.7 (9)	505 (9)
	Accuracy (Bias%)		1.37	1.01
	Precision (CV %)		8.27	5.06
Efavirenz	Overall Mean (n)	5.05 (9)	48.6 (9)	480 (9)
	Accuracy (Bias%)	0.88	-2.86	-3.99
	Precision (CV %)	5.59	2.66	1.89
8-OH-efavirenz	Overall Mean (n)	5.31 (9)	50.0 (9)	445 (9)
	Accuracy (Bias%)	6.10	0	-10.9
	Precision (CV %)	4.58	5.00	2.35

Supplementary Table 2: Calibration Ranges

Analyte	LLOQ plasma (ng/mL)	ULOQ plasma (ng/ml)
Metoprolol	1	1000
OH-metoprolol	0.5	1000
Omeprazole	0.5	1000
5-OH-omeprazole	0.5	1000
Losartan	1.0	1000
E3174	1.0	1000
Midazolam	0.5	1000
1'-OH-midazolam	0.5	1000
Caffeine	10.0	1000
Paraxanthine	10.0	1000
Efavirenz	0.5	1000
8-OH-efavirenz	1.0	1000

Supplementary Table 3: Autosampler Stability (24 hours at 10°C)

nominal concentrations		QC1 = 5 ng/mL	QC2 = 50 ng/mL	QC3 = 500 ng/mL
Metoprolol	Overall Mean t=0 (n=3)	5.55	49.9	484
	Overall Mean t=24h (n=3)	5.70	47.1	469
	Stability	**103**	**94**	**97**
OH-metoprolol	Overall Mean t=0 (n=3)	4.74	50.4	479
	Overall Mean t=24h (n=3)	4.84	45.7	444
	Stability	**102**	**91**	**93**
Omeprazole	Overall Mean t=0 (n=3)	5.05	51.9	478
	Overall Mean t=24h (n=3)	5.13	49.8	476
	Stability	102	96	100
5-OH-omeprazole	Overall Mean t=0 (n=3)	4.87	50.8	496
	Overall Mean t=24h (n=3)	5.32	49.3	492
	Stability	**109**	**97**	**99**
Losartan	Overall Mean t=0 (n=3)	5.04	51.1	484
	Overall Mean t=24h (n=3)	5.10	50.0	495
	Stability	**101**	**98**	**102**
E-3174	Overall Mean t=0 (n=3)	4.76	49.1	479
	Overall Mean t=24h (n=3)	5.11	52.2	515
	Stability	**107**	**106**	**108**
Midazolam	Overall Mean t=0 (n=3)	4.79	50.0	481
	Overall Mean t=24h (n=3)	4.86	52.3	495
	Stability	**101**	**105**	**103**
1'-OH-midazolam	Overall Mean t=0 (n=3)	4.64	47.7	484
	Overall Mean t=24h (n=3)	4.83	49.9	497
	Stability	**104**	**105**	**103**
Caffeine	Overall Mean t=0 (n=3)		50.4	495
	Overall Mean t=24h (n=3)		55.0	493

	Stability		**109**	**100**
Paraxanthine	Overall Mean t=0 (n=3)	4.59	49.6	483
	Overall Mean t=24h (n=3)	5.27	52.1	498
	Stability	**114.80**	**104.9**	**103**
Efavirenz	Overall Mean t=0 (n=3)		50.4	486
	Overall Mean t=24h (n=3)		50.1	515
	Stability		**100**	**106**
8-OH-efavirenz	Overall Mean t=0 (n=3)	5.04	50.8	487
	Overall Mean t=24h (n=3)	4.72	49.3	508
	Stability	**94**	**97**	**104**

Supplementary Table 4: PK parameters of probe drugs and metabolites

		AUC[1] (ng/ml*h)	point estimate and 90%CI for AUC ratio from BE test	C_{max} (ng/ml)	halflife (h)
Caffeine	baseline	7507 (6656-9469)	-	1088±264	5.0±2.0
	inhibition	12146 (10823-14836)	162 (140 – 187)	1129±277	7.8±2.8
	induction	5761 (5145-7052)	78 (67-91)	1046±203	3.7±1.6
Paraxanthine	baseline	4695 (4093-5930)	-	338±110	9.2± 4.9
	inhibition	4620 (4002-6135)	98 (86-113)	297±135	22.0±14.1
	induction	3656 (3267-4392)	80 (71-90)	340±76	7.1±4.5
Efavirenz	baseline	2780 (2611-3025)	-	247±73	61.7±16.5
	inhibition	2741 (2601-2935)	99 (92-106)	222±51	61.6±17.2
	induction	1709 (1593-1876)	61 (57-65)	192±62	40.5±12.9
OH-efavirenz	baseline	67 (61-78)	-	21.2±38.8	78.4±41.9
	inhibition	37 (34-44)	56 (51-61)	4.1±1.5	178.4±196.0
	induction	81 (74-92)	116 (109-124)	15.8±6.6	51.4±69.4
losartan	baseline	33 (30-38)	-	10.4±5.5	3.1±1.0
	inhibition	40 (35-49)	120 (106-135)	8.6±4.0	4.2±1.6
	induction	14 (13-16)	61 (57-65)	7.5±12.3	4.4±6.0
Losartan carb acid	baseline	390 (363-523)	-	39.0±16.8	6.0±1.5
	inhibition	168 (154-254)	43 (37-51)	12.2±6.0	11.7±5.6
	induction	201 (185-262)	52 (46-59)	30.6±17.0	4.0±0.8
omeprazole	baseline	186 (156-269)	-	132.2±91.3	0.81±0.43
	inhibition	2511 (2315-2809)	1342 (1112-1620)	453.1±49.8	3.06±0.54
	induction	13 (11-22)	6.8 (5.4-8.6)	18.9±39.1	0.56±0.26
OH-omeprazole	baseline	210 (196-230)	-	85.6±23.1	1.14±0.25
	inhibition	140 (131-153)	67 (59-76)	19.8±5.5	4.07±0.76
	induction	60 (53-70)	28 (25-32)	32.0±10.3	0.92±0.71
metoprolol	baseline	104 (98-112)	-	6.7±1.5	17.8±12.3
	inhibition	213 (201-230)	207 (188 – 227)	12.0±1.8	38.5±35.7
	induction	88 (82-96)	86 (79 – 93)	5.9±1.4	14.2±6.8
OH-metoprolol	baseline	61 (57-69)	-	3.4±0.9	29.9±20.4
	inhibition	28 (23-36)	40 (33-49)	1.3±0.6	19.8±10.5
	induction	61 (55-70)	98 (93-104)	3.5±1.0	26.0±16.2
midazolam	baseline	24 (22-30)	-	11.4±4.0	2.1±0.7
	inhibition	98 (90-114)	409 (350-478)	28.3±6.3	4.3±1.9
	induction	3.8 (3.4-5.2)	15 (11-20)	3.4±1.4	0.9±0.2
1-OH-midazolam	baseline	16 (14-19)	-	8.1±3.6	1.59±0.46
	inhibition	22 (20-27)	143 (121-168)	7.7±3.5	2.54±0.93
	induction	1.0 (0.9-1.3)	6.3 (4.7-8.3)	1.3±0.6	n.d.
1-OH-midazolam[2]	baseline	151 (141-167)	-	58.6±17.2	2.0±0.7
	inhibition	160 (147-181)	106 (102-111)	36.4±10.2	2.8±0.5
	induction	106 (97-120)	71 (67-75)	51.7±15.5	1.2±0.4

AUC data are given as geometric mean (90% CI), Cmax and half-life data are given as mean±sd. n.d., not determined due to insufficient data above LLOQ

[1] AUClast for losartan, losartan carboxylic acid, omeprazole, 5-hydroxyomeprazole, midazolam, 1-hydroxymidazolam, AUC24 for all other analytes

[2] total 1-hydroxymidazolam concentration after deglucuronidation with beta-glucuronidase

Supplementary Table 5: Pharmacogenetic Analysis

CYP	*Allele*	Subj 1	Subj 2	Subj 3	Subj 4	Subj 5	Subj 6	Subj 7	Subj 9	Subj 10	Subj 11	Subj 12	Subj 13	Subj 14	Subj 15	Subj 16
1A2	*CYP1A2*1F*	HT	HT	HO	HT	WT	HT	HT	HO	HO	HT	HT	HT	HT	HT	HO
2B6	*CYP2B6*6*	HT	WT	WT	HT	WT	HT	HT	HT	WT	HT	HT	HT	HT	WT	WT
2C9	*CYP2C9*2*	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	HT	WT	WT
	*CYP2C9*3*	WT	WT	WT	HT	HT	WT	HT	WT	WT	WT	HT	HT	WT	WT	WT
2C19	*CYP2C19*2*	WT	WT	WT	WT	WT	HT	WT	HT	HT	WT	WT	HT	WT	HO	WT
	*CYP2C19*3*	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
2D6	*CYP2D6*3*	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	*CYP2D6*4*	WT	HT	HT	HT	HT	WT	WT	WT	HT	WT	HT	WT	WT	WT	HT
	*CYP2D6*5*	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	*CYP2D6*6*	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	*CYP2D6*XN*	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	*CYP2D6*41*	nd	nd	nd	nd	nd	nd	nd	nd	HT	nd	nd	nd	nd	nd	nd

HO, homozygous; HT, heterozygous; nd, not done; neg, no gene multiplication observed; WT, wild type. Subject 8 did not provide consent for pharmacogenetic analysis. *CYP2D6**41 was only tested in subject 10.