SUPPLEMENTAL DATA

Cytochrome P450 2C19 phenoconversion by routinely prescribed proton pump inhibitors omeprazole and esomeprazole: clinical implications for personalized medicine

Martin Klieber, MSc; Herbert Oberacher, PhD; Silvia Hofstaetter, Beate Beer, MD; Martin Neururer, BSc; Anton Amann, PhD; Hannes Alber, MD*; and Anil Modak, PhD*

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Experimental

Chemicals and oligonucleotides

Acetonitrile (HPLC gradient grade) was obtained from Sigma-Aldrich (Schnelldorf, Germany). A 1.0 M stock solution of cyclohexyldimethylammonium acetate (CycHDMMA) was prepared by titration of cyclohexyldimethylamine (Sigma-Aldrich) with acetic acid (Sigma-Aldrich) at 5°C until pH 8.4 was reached. For preparation of all solutions, HPLC grade water (Sigma-Aldrich) was used. Oligonucleotides were purchased from Microsynth (Balgach, Switzerland).

DNA extraction

DNA was extracted from saliva swabs or blood using the Chelex method (Ward 1991).

Amplification of 1.9 kb fragment covering exons 4 and 5

The long-PCR and thermocycler protocol was adopted from Kisko et al. with slight modifications (Koski 2006). To isolate the CYP2C19 gene from the flanking highly homologous pseudogenes, a 1.9 kb fragment covering exons 4 and 5 was amplified using the primers CYP2C19-F (5'-GCTAGGCTGTAATTGTTAATTCGA-3') and CYP2C19-R (5'-TTACATTTTCTATGATGCTTACTGGA-3'). Each reaction had a final volume of 21 µl and contained 0.6 U of GeneAmp rTth DNA Polymerase XL (Applied Biosystems, Foster City, USA), 1 x XL Buffer II, 5 µg bovine serum albumin, 0.25 mM of each dNTP, 1.25 mM $Mg(OAc)_2$, 0.4 µM each primer, and 4 µl DNA extract. Amplification was carried out in a Gene Amp PCR System 9700 (Applied Biosystems) according to following protocol: initial denaturation step at 94 °C for 1 min, 10 cycles of 94 °C for 30 s and 60 °C for 10 min, 25 cycles of 94 °C for 30 s and 60 °C for 10 min and 15 s plus 15 s per cycle and a final extension step at 72 °C for 30 min.

*Amplification of the CYP2C19 alleles *2 (rs4244285), *3 (rs4986893), and *17 (rs12248560)*

The following primers were used to amplify an 86 bp-long product containing rs4244285: 5'-TTGTTTTCTCTTAGATATGCAATAA-3' and 5'-AGCAAGGTTTTTAAGTAATTTGT-3'.

The following primers were used to amplify an 76 bp-long product containing rs4986893: 5'-TTGAATGAAAACATCAGGATTGTA-3' and 5'-GTGGTTTCTCAGGAAGCAAAA-3'. The following primers were used to amplify an 57 bp-long product containing rs12248560: 5'-CAAATTTGTGTCTTCTGTTCTCAAA-3' and 5'-TCGTGGCGCATTATCTCTTA-3'. The 20 µl reaction mixture contained 1x AmpliTaq Gold PCR Buffer II (Applied Biosystems), 1.9 mM $MgCl_2$, 2 µl DNA extract (rs12248560) or 2 µl of the diluted (1:1000 with sterile water) product of the long-PCR (rs4244285, rs4986893), 1.0 µM of each primer, 1 unit AmpliTaq Gold Polymerase (Applied Biosystems) and 0.25 mM of each dNTP. Amplification was carried out in a Gene Amp PCR System 9700 (Applied Biosystems) by an initial denaturation step of 95 °C for 10 min, 40 cycles of 94 °C for 30 s, 55 °C for 45 s and 72 °C for 30 s, and final extension at 72°C for 10 min.

Ion-pair reversed-phase high-performance liquid chromatography-electrospray ionization mass spectrometry (ICEMS)

An Ultimate fully integrated capillary HPLC system (LC-Packings, Amsterdam, The Netherlands) was used for all chromatographic experiments. A Famos autosampler (LC42 Packings) equipped with a 2 µl loop was used for sample injection. The 45 x 0.2 mm i.d. monolithic capillary column was prepared according to the published protocol (Premstaller 2000). The flow rate was set to 2.0 µl/min. The column temperature was set to 70 °C to denature the amplicons into the corresponding single-strands. The PCR amplicons were injected onto the column without any prior sample preparations step. Separation of the single-stranded DNA molecules was accomplished with a gradient of 5-50% acetonitrile in 25 mM CycHDMAA within 15 min. The eluting nucleic acids were detected online by ESI-MS, which was performed on a QSTAR XL mass spectrometer (Applied Biosystems) equipped with a modified TurboIonSpray source (Oberacher 2005). Mass calibration and optimization of instrumental parameters were performed in the negative ion mode as described previously (Oberacher 2005). The spray voltage was set to 3.8 kV. Gas flows of 35 arbitrary units (nebulizer gas) and 25 arbitrary units (turbo gas) were employed. The temperature of the turbo gas was adjusted to 200 °C. The accumulation time was set to 1 s, and 10 time bins were summed up. Mass spectra were recorded in the range between 800 and 1200 on a personal computer operating with the Analyst QS software (1.0, service pack 8 and Bioanalyst extension, Applied Biosystems).

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Table legends

Table 1 ; Sex, age, weight, height, genotype, phenotype, genotype-phenotype discordance characteristics and PPI administered (BT- before PPI therapy; AT – after PPI therapy; AS - Activity score based on genotype)

Table 2. Interday intrapatient variation in CYP2C19 phenotype (DOB_{30}) determined by Ptz-BT in 31 volunteers/patients on 2 separate visits 1 week apart.

Table 3 DOB values for the Ptz-BT for two visits, Cmax and Tmax of volunteers/patients (n=31) to determine the single time point breath collection for CYP2C19 phenotype using Cmax and Tmax

Table 1: Sex, age, weight, height, genotype, phenotype, genotype-phenotype discordance characteristics and PPI administered (BT- before PPI therapy; AT – after PPI therapy; AS - Activity score based on genotype)

ID	Sex	Age	Wt kg	Ht m	Avg DOB_{30} BT	DOB_{30} AT	AS	Genotype	Phenotype from genotype	*Phenotype from DOB_{30} BT	*Phenotype from DOB_{30} AT	Geno-Pheno discordance BT	Geno-Pheno discordance AT	Pheno-conversion BT-AT	Study medication (40mg)
6	m	27	100	1.79	3.5	0.3	3	*1/*17	UM	EM	PM	Yes	Yes	Yes	Esomeprazole
8	m	28	86	1.86	3.7	1.0	3	*1/*17	UM	EM	PM	Yes	Yes	Yes	Esomeprazole
9	m	25	90	1.88	4.5	3.0	3	*1/*17	UM	EM	IM	Yes	Yes	Yes	Omeprazole
10	m	26	85	1.87	4.2	3.9	3	*1/*17	UM	EM	EM	Yes	Yes	No	Esomeprazole
11	f	19	85	1.67	4.3	1.8	3	*1/*17	UM	EM	IM	Yes	Yes	Yes	Omeprazole
26	f	18	60	1.7	7.0	1.9	3	*1/*17	UM	EM	IM	Yes	Yes	Yes	Esomeprazole
57	m	51	82	1.76	6.4	2.0	3	*1/*17	UM	EM	IM	Yes	Yes	Yes	Esomeprazole
1	m	38	94	1.78	4.1	0.7	2	*1 /*1	EM	EM	PM	No	Yes	Yes	Omeprazole
2	m	25	70	1.7	3.9	1.5	2	*1/*1	EM	EM	IM	No	Yes	Yes	Esomeprazole
12	f	23	55	1.65	6.5	2.1	2	*1/*1	EM	EM	IM	No	Yes	Yes	Esomeprazole
17	m	19	88	1.83	4.0	1.5	2	*1/*1	EM	EM	IM	No	Yes	Yes	Omeprazole
22	m	23	65	1.76	6.5	1.1	2	*1/*1	EM	EM	PM	No	Yes	Yes	Esomeprazole
23	f	22	57	1.63	6.2	2.3	2	*1/*1	EM	EM	IM	No	Yes	Yes	Omeprazole
52	m	54	85	1.8	4.4	1.2	2	*1/*1	EM	EM	IM	No	Yes	Yes	Omeprazole
3	f	23	75	1.8	3.0	0.6	2	*1/*1	EM	IM	PM	Yes	Yes	Yes	Omeprazole
5	m	24	120	1.85	1.4	1.0	2	*1/*1	EM	IM	PM	Yes	Yes	Yes	Omeprazole
7	m	23	73	1.82	2.9	1.1	2	*2/*17	EM	IM	PM	Yes	Yes	Yes	Omeprazole
19	f	26	60	1.65	2.8	0.3	2	*1/*1	EM	IM	PM	Yes	Yes	Yes	Omeprazole
54	m	49	81	1.77	2.9	1.0	2	*1/*1	EM	IM	PM	Yes	Yes	Yes	Omeprazole
15	f	26	76	1.58	6.1	0.9	1	*1/*2	IM	EM	PM	Yes	Yes	Yes	Omeprazole
18	m	23	65	1.78	4.0	0.1	1	*1/*2	IM	EM	PM	Yes	Yes	Yes	Esomeprazole
21	m	22	72	1.82	4.9	1.2	1	*1/*2	IM	EM	IM	Yes	No	Yes	Omeprazole
56	m	49	104	1.84	3.7	1.2	1	*1/*2	IM	EM	IM	Yes	No	Yes	Omeprazole
4	f	26	54	1.67	1.7	0.3	1	*1/*2	IM	IM	PM	No	Yes	Yes	Esomeprazole
13	f	25	68	1.68	2.6	0.0	1	*1/*2	IM	IM	PM	No	Yes	Yes	Omeprazole
16	m	25	112	1.7	1.5	0.1	1	*1/*2	IM	IM	PM	No	Yes	Yes	Esomeprazole
24	f	33	50	1.62	0.6	0.9	1	*1/*2	IM	PM	PM	Yes	Yes	na	Esomeprazole
25	f	21	70	1.7	1.0	0.5	1	*1/*2	IM	PM	PM	Yes	Yes	na	Omeprazole
53	m	54	73	1.78	0.9	0.8	1	*1/*2	IM	PM	PM	Yes	Yes	na	Esomeprazole
20	m	28	80	1.79	0.2	-0.1	0	*2/*2	PM	PM	PM	No	No	na	Esomeprazole
55	m	48	80	1.79	0.5	0.0	0	*2/*2	PM	PM	PM	No	No	na	Esomeprazole

*** EM 3.5-7.0 ‰; IM 1.2-3.5 ‰; PM <1.2 ‰; UM >7.0**

Table 2. Interday intrapatient variation in CYP2C19 phenotype (DOB_{30}) determined by Ptz-BT in 31 volunteers/patients on 2 separate visits 1 week apart.

No.	Visit 1 DOB_{30} in ‰	Visit 2 DOB_{30} in ‰	Avg DOB_{30} in ‰	SD	CV	Change in ‰ Visit 1 & 2
1	4	4.2	4.1	0.1	0.02	0.2
2	4	3.7	3.9	0.2	0.05	0.3
3	3	2.9	3.0	0.1	0.03	0.3
4	1.3	2	1.7	0.5	0.29	0.7
5	1.2	1.5	1.4	0.2	0.14	0.3
6	3.6	3.3	3.5	0.2	0.05	0.3
7	3.5	2.3	2.9	0.8	0.27	1.2
8	4	3.3	3.7	0.5	0.13	0.7
9	3.5	5.5	4.5	1.4	0.31	2.2
10	4.4	3.9	4.2	0.4	0.09	0.5
11	4.2	4.4	4.3	0.1	0.02	0.2
12	4.6	8.4	6.5	2.7	0.41	3.8
13	3.2	2	2.6	0.8	0.30	1.2
15	6.1	6	6.1	0.1	0.02	0.1
16	1.8	1.2	1.5	0.4	0.26	0.6
17	4	4	4.0	0.0	0.00	0
18	3.5	4.4	4.0	0.6	0.15	0.9
19	2.9	2.6	2.8	0.2	0.07	0.3
20	0.1	0.2	0.2	0.1	0.50	0.1
21	5.7	4.1	4.9	1.1	0.22	1.6
22	7.2	5.7	6.5	1.1	0.16	1.5
23	6.1	6.3	6.2	0.1	0.02	0.2
24	0.8	0.4	0.6	0.3	0.50	0.4
25	0.6	1.3	1.0	0.5	0.50	0.7
26	8.2	5.8	7.0	1.7	0.24	2.4
52	4.5	4.2	4.4	0.2	0.05	0.3
53	0.9	0.8	0.9	0.1	0.11	0.1
54	3	2.7	2.9	0.2	0.07	0.3
55	0.5	0.4	0.5	0.1	0.20	0.1
56	3.7	3.7	3.7	0.0	0.00	0
57	6.3	6.4	6.4	0.1	0.02	0.1
Average	**3.56**	**3.47**	**3.52**	**0.5**	**0.17**	**0.70**

Table 3 DOB values for the Ptz-BT for two visits, Cmax and Tmax of volunteers/patients (n=31) to determine the single time point breath collection for CYP2C19 phenotype using Cmax and Tmax

ID	Visit	DOB_{20}	DOB_{30}	DOB_{40}	Cmax	Tmax in mins
1	1	**4.3**	4	3.8	4.3	20
1	2	4.4	4.2	**5.1**	5.1	40
2	1	2.9	**4**	3.7	4	30
2	2	3.2	**3.7**	3.3	3.7	30
3	1	2.6	3	**3.2**	3.2	40
3	2	2.5	2.9	**3**	3	40
4	1	1.3	1.3	**1.6**	1.6	40
4	2	1.3	**2**	1.2	2	30
5	1	0.3	**1.2**	0.4	1.2	30
5	2	**1.7**	1.5	0.6	1.7	20
6	1	**3.9**	3.6	3.7	3.9	20
6	2	**4.8**	3.3	2.7	4.8	20
7	1	3.3	**3.5**	**3.5**	3.5	30
7	2	2.2	**2.3**	2.2	2.3	30
8	1	3.8	**4**	3.1	4	30
8	2	**4.5**	3.3	4.2	4.5	20
9	1	**3.9**	3.5	3	3.9	20
9	2	**6.4**	5.5	4.6	6.4	20
10	1	3.4	**4.4**	**4.4**	4.4	30
10	2	**4.3**	3.9	3.4	4.3	20
11	1	**4.4**	4.2	**4.4**	4.4	20
11	2	5	**4.4**	**4.4**	4.4	30
12	1	2.8	**4.6**	4.3	4.6	30
12	2	**8.5**	8.4	6.8	8.5	20
13	1	2.1	**3.2**	2.8	3.2	30
13	2	1.7	**2**	**2**	2	30
15	1	5.7	**6.1**	**6.1**	6.1	30
15	2	5.7	**6**	**6**	6	30

16	1	1.4	**1.8**	1.4	1.8	30
16	2	1.1	1.2	**1.3**	1.3	40
17	1	**4.2**	4	3.9	4.2	20
17	2	**4.4**	4	3.3	4.4	20
18	1	3.9	3.5	**4.8**	4.8	40
18	2	4.0	**4.4**	4.2	4.4	30
19	1	2.6	**2.9**	2.7	2.9	30
19	2	**2.6**	**2.6**	2.1	2.6	30
20	1	-0.7	0.1	**0.2**	0.2	40
20	2	0	0.2	**0.4**	0.4	40
21	1	5.4	**5.7**	5	5.7	30
21	2	**5.2**	4.1	3.4	5.2	20
22	1	5.6	**7.2**	5.8	7.2	30
22	2	4.4	5.7	**6.3**	6.3	40
23	1	5.6	**6.1**	5.6	6.1	30
23	2	6.4	6.3	**6.8**	6.8	40
24	1	0.4	0.8	**1.1**	1.1	40
24	2	**0.4**	**0.4**	0.2	0.4	20
25	1	**0.8**	0.6	0.4	0.8	20
25	2	1.1	**1.3**	**1.3**	1.3	30
26	1	**8.3**	8.2	7.6	8.3	20
26	2	2.7	5.8	**6.6**	6.6	40
52	1	**4.7**	4.5	4.5	4.7	20
52	2	**4.3**	4.2	4.2	4.3	20
53	1	0.5	0.9	**1.3**	1.3	40
53	2	**1**	0.8	**1**	1	20
54	1	**3.3**	3	2.9	3.3	20
54	2	**2.9**	2.7	2	2.9	20
55	1	0.1	**0.5**	0	0.5	30
55	2	0.5	0.4	**0.7**	0.7	40
56	1	3.4	**3.7**	3.3	3.7	30

56	2	**4.2**	3.7	3.5	4.2	20

57	1	**8.6**	6.3	6	8.6	20
57	2	**7.2**	6.4	6.5	7.2	20
Average		**3.4**	**3.5**	**3.4**	**3.8**	**28.4**