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Nutrimetric Validation of Solanidine as Dietary-Derived CYP2D6 Activity Marker In Vivo

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CYP2D6 is involved in the metabolism of many drugs. Its activity is affected by pharmacogenetic variability leading to highly polymorphic phenotypes between individuals, affecting safety and efficacy of drugs. Recently, solanidine, a steroidal alkaloid from potatoes, and its metabolites, has been identified as a dietary-derived activity marker for CYP2D6. The intraday variability in plasma within individuals has not been studied yet in healthy subjects. As part of a CYP phenotyping cocktail study with 20 healthy participants, plasma concentrations of solanidine, 4-OH-solanidine and 3,4-secosolanidine-3,4-dioic acid (SSDA) were determined using a sensitive liquid chromatography-mass spectrometry method in urine and in plasma at timepoints 0, 2.5, 5, 8, and 24 hours after intake of test substances. The participants were phenotyped for CYP2D6 with oral metoprolol (12.5 mg) with 15 plasma sampling points over 24 hours (DRKS00028922). Metabolic ratios (MRs) of metabolite to parent plasma concentrations were formed from single timepoints and the area under the curve (AUC). All participants were genotyped for CYP2D6. The intra-individual variability of the CYP2D6 metabolite SSDA was highly stable with a median SD of 11.62% over 24 hours. MR SSDA/solanidine was more variable (median SD 31.90%) but correlated significantly at all measured timepoints with AUC MR α-OH-metoprolol/metoprolol. The AUC MR SSDA/solanidine showed a significant linear relationship with the genetically predicted CYP2D6 activity score. This study substantiates the MR SSDA/solanidine as CYP2D6 activity marker. The high correlation with metoprolol MR indicates a valid prediction of the CYP2D6 phenotype at any timepoint during the study day.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Recently, solanidine and its metabolites were described as novel CYP2D6 biomarkers, but little is known on their nutrimetrics and variation over the day.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ As part of a cocktail study plasma concentrations of solanidine and its metabolites were measured at 0, 2.5, 5, 8, and 24 hours to assess variability of analyte signals and corresponding metabolic ratios (MRs). Correlation of solanidine MR to metoprolol MR and the relationship to the CYP2D6 activity score was assessed.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Analyte levels of 3,4-secosolanidine-3,4-dioic acid (SSDA) were the most stable during the day, with a median SD of 11.62%. The MR SSDA/solanidine correlated significantly with MR α-OH-metoprolol/metoprolol, even after exclusion of poor metabolizers. In addition, a significant linear relationship with the CYP2D6 activity score was shown.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The plasma MR SSDA/solanidine is a valid phenotyping probe for CYP2D6. Assessment of SSDA/solanidine in plasma could replace administration of a CYP2D6 probe drug, which may not be appropriate in vulnerable patients.

Almost 20–30% of all clinically used drugs are metabolized by the enzyme CYP2D6.[1,2] CYP2D6 is particularly important in the metabolism of several psychiatric drugs, such as antidepressants and beta-blockers like metoprolol, but is also involved in the activation of prodrugs like tamoxifen or the opioid analgesics codeine and tramadol.[1] CYP2D6 is highly

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polymorphic, demonstrating a broad range of activities and interindividual variability.[2] Hence, individuals can show highly variable exposure to CYP2D6 metabolized drugs affecting safety and efficacy of the treatment.

Although the metabolic activity can be somewhat predicted from the genotype, variation in activity due to drug–drug interactions cannot be assessed, which is an inherent limitation of genotyping.[3] In addition, the influence of rare variants is seldom assessed and therefore, only 40% of the variability in CYP2D6 activity is explained by common genotypes.[4,5]

Hence, precise phenotyping methods are needed to determine CYP2D6 activity and enable personalized drug selection and dosing regimens. Commonly, phenotyping probe drugs are administered in research or clinical studies.[6,7] One specific probe drug for CYP2D6 is metoprolol, which is often used in cocktail studies, and specifically metabolized by CYP2D6 to α-OH-metoprolol. The administration of an external probe drug like metoprolol is impractical and carries the risk of adverse effects in many clinical situations. Hence, it is difficult to apply in patients, particularly in the old and poly-medicated; although these patient groups would highly benefit from it.

To circumvent these limitations, research focused on endogenous or dietary activity markers.[8] It would be of value to validate biomarkers that can be analyzed in plasma or urine of patients without external administration of CYP2D6 probe drugs and plasma sampling at defined timepoints.

In a previous study, an untargeted metabolomics approach revealed the compound solanidine,[9] a steroidal alkaloid present in potatoes and other nightshades,[10,11] and 5 of its metabolites as potential CYP2D6 biomarkers in healthy volunteers receiving CYP2D6 inhibition by paroxetine.[9] A marked decrease of solanidine metabolites in human plasma and urine after paroxetine treatment was observed indicating that solanidine may serve as a valuable CYP2D6 probe drug measuring enzyme inhibition.[9] These results were verified by the correlation of the solanidine metabolic ratio (MR) to the urinary MR of dextrorphan/dextromethorphan.[9]

One of the identified solanidine metabolites corresponds to the previously identified urinary CYP2D6 biomarker M1 (m/z 444.3102) by Tay-Sontheimer and colleagues.[12] Its structure was recently solved and identified as 3,4-secosolanidine-3,4-dioic acid (SSDA), using an extraction from urine, mass spectrometry, and nuclear magnetic resonance approaches.[13] A further solanidine metabolite of the mass 414.3366 m/z was identified as 4-OH-solanidine, and is proposed to be the first metabolite generated by CYP2D6 in the pathway toward the formation of SSDA.[13]

In a retrospective analysis with high-resolution mass spectrometry data of therapeutic drug monitoring from CYP2D6 genotyped patients, the MR of 4-OH-solanidine/solanidine had a 100% accuracy in detecting CYP2D6 poor metabolizer (PM).[14] Another study from the same group showed significant correlations of the MR 4-OH-solanidine/solanidine and SSDA/solanidine to the MR 9-hydroxyrisperidone/risperidone, which is mainly metabolized by CYP2D6.[15]

For further validation of solanidine as CYP2D6 phenotyping probe, increased knowledge of nutrikinetic parameters would be important. Because neither the half-life nor the absorption properties of solanidine and its metabolites are known, we aimed to assess within-subject variability of solanidine concentrations during the day, as well as concentrations of the metabolites in comparison to the CYP2D6 phenotype measured by metoprolol MR.

METHODS

Study design

Samples were analyzed from a clinical CYP phenotyping cocktail study in healthy volunteers that was performed for another purpose in the Institute for Occupational, Social and Environmental Medicine, University Hospital RWTH Aachen. Twenty healthy subjects gave written informed consent prior to recruitment to the study. The study was approved by the ethical committee of the RWTH Aachen and registered in the German Clinical Trials Register under the No.: DRKS00028922. The study was conducted in accordance with the Declaration of Helsinki. Subjects were administered a single dose oral drug cocktail of CYP phenotyping drugs to assess the activities of CYP1A2/CYP2A6 (caffeine), CYP3A4 (midazolam), CYP2C9 (torsemide), CYP2C19 (omeprazole), CYP2B6 (efavirenz), and CYP2D6 (12.5 mg metoprolol).[4,6,7] On the study day, subjects were fasting until 3 hours after drug cocktail intake, after which a meal was provided. Afterward, subjects were free to drink water, juice, or tea (but no caffeinated beverages). An evening meal and a breakfast were provided the next day. A manuscript including a detailed description of the study design is in preparation. Blood sampling was performed over 24 hours with 15 timepoints at 0, 0.25, 0.5, 0.45, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, and 24 hours after drug cocktail intake. Urine was collected and weighed during the study.

Pharmacogenetic analysis

Pharmacogenetic characterization of CYP2D6 variants, including *3, *4, *6, *7, *8, *9, *10, *14, *17, and *41 was performed according to a customized pharmacogenetic genotyping panel using TaqMan OpenArray Real-Time PCR system (ThermoFisher, Waltham, MA). CYP2D6 deletions (*5) and gene duplications were assessed using the TaqMan copy number assay Hs00010001_cn (ThermoFisher). Genotyping was performed at the Dr. Margarete Fischer-Bosch-Institute for Clinical Pharmacology, Stuttgart. The results were 100% in concordance with the results of an orthogonal method using Infinium Global Screening Arrays version 2.0 (Illumina, San Diego, CA) with 700 k variants if appropriate probes existed on the chip. Array experiments were performed by LIFE & BRAIN GmbH, Bonn, Germany. The genotypes were translated into CYP2D6 metabolizer phenotypes according to a recent trial[16] and a revised guideline from the Clinical Pharmacogenetics Implementation Consortium (CPIC), with *9, *10, and *41 variants assigned an activity score of 0.25.[17,18]

Material

Solanidine (13264-1MG; Sigma), metoprolol (HY-17503; MedChemExpress), α-OH-metoprolol (Cay28020-1; Biomol), metoprolol-d6 (Cay28188-1; Biomol), α-OH-metoprolol-d5 (TOR-H948392-1MG; Biozol), formic acid (84865.180, HiPerSolv; VWR Chemicals), methanol (ultra-gradient HPLC grade; 8402; J.T. Baker), water (LiChrosolv, liquid chromatography-mass spectrometry (LC–MS) grade, 1.15333.2500; Merck), and bovine serum (2203-010; Acila).

LC–MS analytics

All analyses were performed with an Agilent 1290 Infinity II UHPLC coupled to a SCIEX QTRAP6500+ triple quadrupole mass spectrometer (MS).

Metoprolol and α-OH-metoprolol were quantified in all 15 plasma sampling points by using a validated LC–MS method (manuscript in preparation). Briefly, 20 µL of plasma samples or calibrators and quality controls in bovine serum were protein precipitated by adding 80 µL methanol with

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internal standards metoprolol-d6 and α-OH-metoprolol-d5. Samples were vortexed for 10 seconds and centrifuged for 20 minutes at 17,000 *g* and 4°C. Then, 40 µL of the supernatant was diluted 1:1 in water, briefly vortexed, centrifuged again for 5 minutes at 17,000 *g* and 4°C. Finally, 50 µL of the supernatants were transferred into an LC vial and 5 µL of the samples were injected for analysis.

Sample preparation for solanidine and solanidine metabolites 4-OH-solanidine and SSDA was as follows: 50 µL of plasma or urine were protein precipitated with 200 µL methanol and vortexed for 10 seconds. Samples were centrifuged at 17,000 *g* for 20 minutes at 4°C. The supernatant was diluted 1:1 in 0.2% (v/v) formic acid in water and briefly vortexed. Samples were centrifuged a second time at 17,000 *g* for 5 minutes at 4°C and 100 µL of the supernatants were transferred to LC-vials. Then, 20 µL of the samples were injected for analysis.

Metoprolol, α-OH-metoprolol, solanidine, and metabolites were separated on an Agilent Poroshell 120 EC-C18 column (1.9 µm, 50 × 2.1 mm; 699675-902; Agilent Technologies) with A: 0.1% (v/v) formic acid in water and B: methanol.

Metoprolol and α-OH-metoprolol were measured in a multiplexed method for all cocktail components and metabolites with a total run-time of 8.5 minutes. The flow rate was 0.7 mL/min. The gradient was 5% B from 0 to 0.2 minutes, followed by a linear increase to 30% B at 2 minutes and a stepwise gradual increase to 34% B at 4.8 minutes and 95% B at 6.5 minutes. Then, 95% B was kept until 7.5 minutes, followed by gradual re-equilibration to 5% B at 7.7 minutes, which was kept until 8.5 minutes.

The total run-time of the method for solanidine and metabolites was 6 minutes with a flow rate of 0.6 mL/min. The gradient for solanidine and metabolites was: 5% B from 0 to 0.2 minutes followed by a linear increase to 95% B at 3.4 minutes, which was kept until 4.4 minutes, followed by re-equilibration to 5% B at 4.5 minutes, which was kept until 6 minutes. The retention times for SSDA, 4-OH-solanidine, and solanidine were 2.3, 2.5, and 2.6 minutes, respectively. Representative chromatograms of an intermediate metabolizer (IM) and PM subject are shown in **Figure S1**C.

Detection of the analytes was performed using multiple reaction monitoring (MRM) in positive ion mode. The mass transitions for metoprolol were m/z 268 ➔ 116, 268 ➔ 121, and 274 ➔ 122 for quantifier, qualifier, and internal standard (metoprolol-d6), respectively. MRM transitions for α-OH-metoprolol were m/z 284 ➔ 116, 284 ➔ 56, and 289 ➔ 121 for quantifier, qualifier, and internal standard (α-OH-metoprolol-d5), respectively. The linear quantification range for metoprolol and α-OH-metoprolol was established from 0.25–200 ng/mL with 7 non-zero calibrators and 5 quality controls. Accuracy and precision (coefficient of variation) at all concentration levels of calibrators and quality controls (QCs) were within ±15% over all measured batches (except 0.5 ng/mL QC for α-OH-metoprolol, which was within ±20%).

Solanidine was tuned to the MS by direct infusion using the commercially available standard. MRM transitions for solanidine were m/z 398.3 ➔ 98.1 and 398.3 ➔ 382.3. In common with previous studies, no analytical standards for solanidine metabolites SSDA and 4-OH-solanidine were available.[9,14,15] Hence, product ion spectra were performed to identify both analytes (**Figure S1A,B**). Fragmentations were confirmed by the literature and the MRM transitions were used accordingly.[9,13] The used MRM transitions for SSDA were m/z 444.3 ➔ 98.1, 444.3 ➔ 370.3, 444.3 ➔ 206, and 444.3 ➔ 56.1. MRM transitions for 4-OH-solanidine were m/z 414.3 ➔ 98.1, 414.3 ➔ 382.3, and 414.3 ➔ 398.3.

Only analyte peaks of solanidine and its metabolites with a minimum signal-to-noise ratio of 10 to 1 were integrated.

Due to missing standards for solanidine metabolites, interday reproducibility between different sample batches is limited; hence, solanidine and metabolites were measured at evenly distributed sampling points (0, 2.5, 5, 8, and 24 hours) over the day, which were the maximum amounts of samples that could be processed and measured in a single batch.

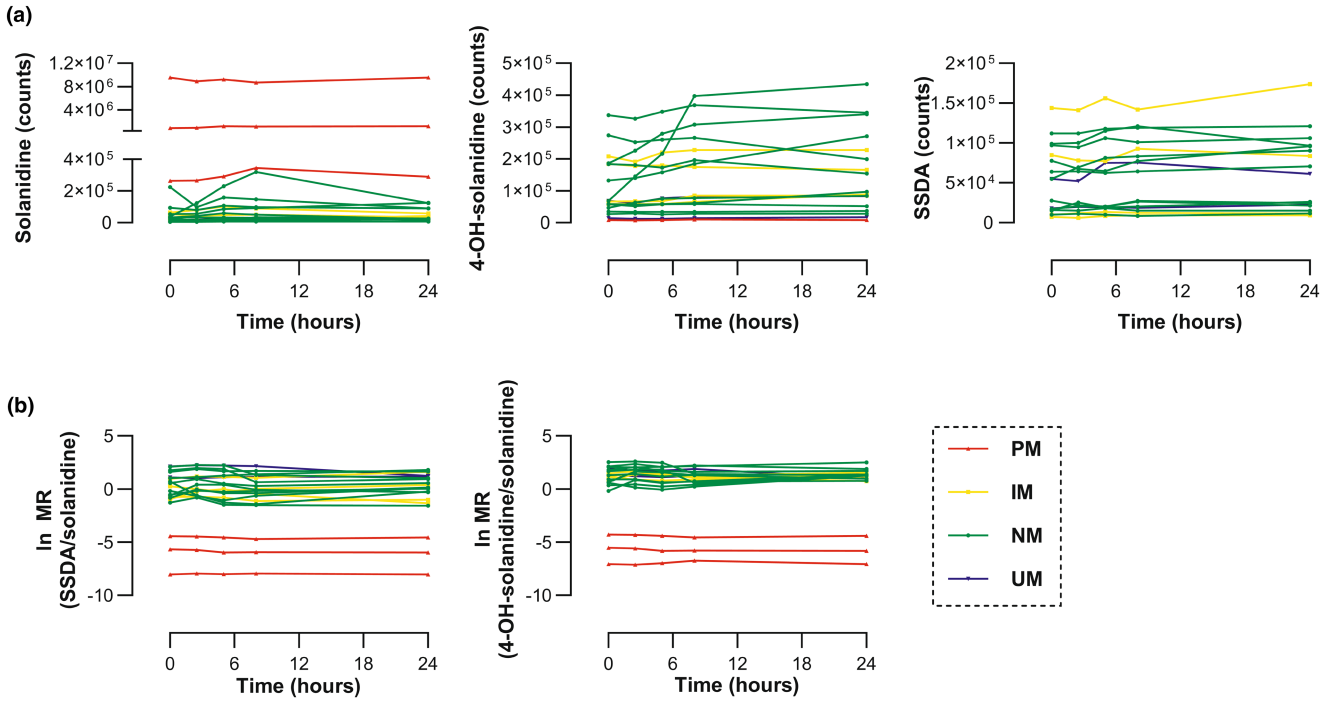

Figure 1 Time-course of analyte signals and metabolic ratios of the subjects allocated to the genotype predicted phenotype. (**a**) Time-course of analyte signals of solanidine, 4-OH-solanidine, and SSDA. (**b**) Time-course of metabolic ratios of SSDA/solanidine and 4-OH-solanidine/solanidine. The time-course for each single subject is shown. IM, intermediate metabolizer; MR, metabolic ratio; NM, normal metabolizer; PM, poor metabolizer; SSDA, 3,4-secosolanidine-3,4-dioic acid; UM, ultrarapid metabolizer.

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Calculation and statistics

Metabolite to solanidine ratios were calculated using the analyte peak area, whereas for metoprolol and α-OH-metoprolol results of the validated quantification method were used. To allow the calculation of MRs, undetectable levels of solanidine and metabolites were truncated to half of the lowest detected value, as previously described.[9,14,15] For two subjects α-OH-metoprolol concentrations were below the limit of quantification at all timepoints, to allow calculation of MR the values were truncated to half of the lowest calculated area under the curve (AUC).

The AUC_{0-8h} for metoprolol and α-OH-metoprolol and AUC_{0-24h} for solanidine and metabolites were calculated with Phoenix WinNonLin (version 8.3) using noncompartmental analysis with linear trapezoidal rule. AUC_{0-8h} for metoprolol and α-OH-metoprolol was used because analyte levels were below the limit of quantification for most subjects at the 24-hour timepoint. MR were calculated as follows: AUC $MR = AUC_{metabolite}/AUC_{parent}$.

Correlations between MR of α-OH-metoprolol/metoprolol and solanidine and its metabolites were calculated with Spearman correlation tests using GraphPad Prism (version 9.3.1). Linear regression for comparison of solanidine and metoprolol MRs to CYP2D6 activity score were performed with GraphPad Prism (version 9.3.1).

Correlations and linear regression with a P value < 0.05 were considered significant.

RESULTS

Solanidine and metabolites were recently identified as novel CYP2D6 biomarkers; however, within-subject variability during the day and the influence of solanidine containing meals on phenotyping is not known. To assess the within-subject variability of solanidine and metabolites in plasma, we measured analyte levels from five sampling points (0, 2.5, 5, 8, and 24 hours) in healthy subjects from the study cohort. From the cohort, 9 subjects were of female and 11 of male sex. The median age (range) was 58.5 (37–73) years and the median body mass index (range) was 26.3 (20.9–37.3) kg/m^2. In each of the 20 subjects across all measured sampling timepoints, we identified the presence of either solanidine or its metabolites (**Figure 1**). Although solanidine was found in every individual within the cohort, it was not necessarily detected at every timepoint. In one subject, solanidine was detected only in one of five (24 hours) and in another subject in two of five (8 and 24 hours) sampling points. In PM subjects, no SSDA metabolite was detected, whereas 4-OH-solanidine was detected in 1 of 3 PM subjects at all sampling timepoints (**Figure 1**). To assess the intra-individual change of analyte levels and MRs over time, the average change (%) compared with the first measured timepoint over 24 hours was calculated for each subject (**Table 1**). For solanidine, 4-OH-solanidine, and SSDA, intraday variability (expressed as the median of the intra-individual SD) was 21.66, 13.20, and 11.62%, respectively. Hence, the analyte that showed the least variation during the 24 hours was SSDA. The MRs of 4-OH-solanidine/solanidine and SSDA/solanidine showed appreciable intraday variation (due to solanidine variation) with median SD of 22.50 and 31.90%, respectively.

Next, we explored the correlation of the AUC_{0-24h} MR of metabolite/solanidine to the AUC_{0-8h} MR of α-OH-metoprolol/metoprolol. The plasma AUC MR 4-OH-solanidine/solanidine correlated significantly with the AUC MR α-OH-metoprolol/metoprolol with a spearman ρ of 0.7158 and a P value of 0.0004 (**Figure 2a**). Interestingly, the AUC MR SSDA/solanidine in plasma showed an even better correlation to AUC MR

Table 1 Intraday and between-subject variability in relative concentrations of solanidine, SSDA, and 4-OH-solanidine and corresponding metabolic ratios over 24 hours

	Genotype-predicted phenotype	PM	IM	NM	UM	All
	Subjects *N*	3	4	11	2	20
Solanidine	Average %	110.40 (96.06–121.76)	105.31 (84.66–129.68)	102.90 (58.74–436.24)	106.96 (100–113.91)	103.87 (58.74–436.24)
	SD %	12.19 (4.16–17.05)	35.48 (14.16–46.19)	23.97 (6.72–284.54)	17.48	21.66 (4.16–284.54)
4-OH-solanidine	Average %	104.4	107.29 (95.51–113.57)	102.43 (91.37–368.84)	102.89 (96.79–109)	103.90 (91.37–368.84)
	SD %	13.36	10.99 (3.77–22.81)	10.83 (4.10–232.39)	14.04 (13.05–15.02)	13.20 (3.77–232.39)
SSDA	Average %	n.d.	107.60 (98.26–118.24)	103.79 (88.33–139.96)	112.69 (109.62–115.77)	105.24 (88.33–139.96)
	SD %	n.d.	11.07 (7.16–17.66)	11.62 (3.75–29.82)	14.91 (9.84–19.99)	11.62 (3.75–29.82)
4-OH-solanidine/solanidine	Average %	91.40 (83.52–109.05)	103.19 (89.27–143.18)	102.44 (72.63–256.29)	88.67 (80.64–96.71)	97.60 (72.63–256.29)
	SD %	12.38 (9.33–17.09)	22.50 (17.21–45.69)	35.48 (8.21–125.75)	18.74 (13.47–24.01)	22.50 (8.21–125.75)
SSDA/solanidine	Average %	83.52 (81.46–104.26)	109.02 (84.89–160.46)	102.96 (38.22–197.68)	97.11 (92.76–101.46)	99.76 (38.22–197.68)
	SD %	12.38 (4.56–12.44)	28.55 (16.32–77.23)	36.15 (12.49–84.46)	17.08 (5.16–28.99)	31.90 (4.56–84.46)

Note: Subjects were grouped into their genotype-predicted phenotype. For each analyte and phenotype group, the average change (%) and standard deviation (SD) (%) over 24 hours is shown with median and the range depicted in brackets. SSDA was not detected in PM subjects.

Abbreviations: IM, intermediate metabolizer; n.d., not detected; NM, normal metabolizer; PM, poor metabolizer; SSDA, 3,4-secosolanidine-3,4-dioic acid; UM, ultrarapid metabolizer.

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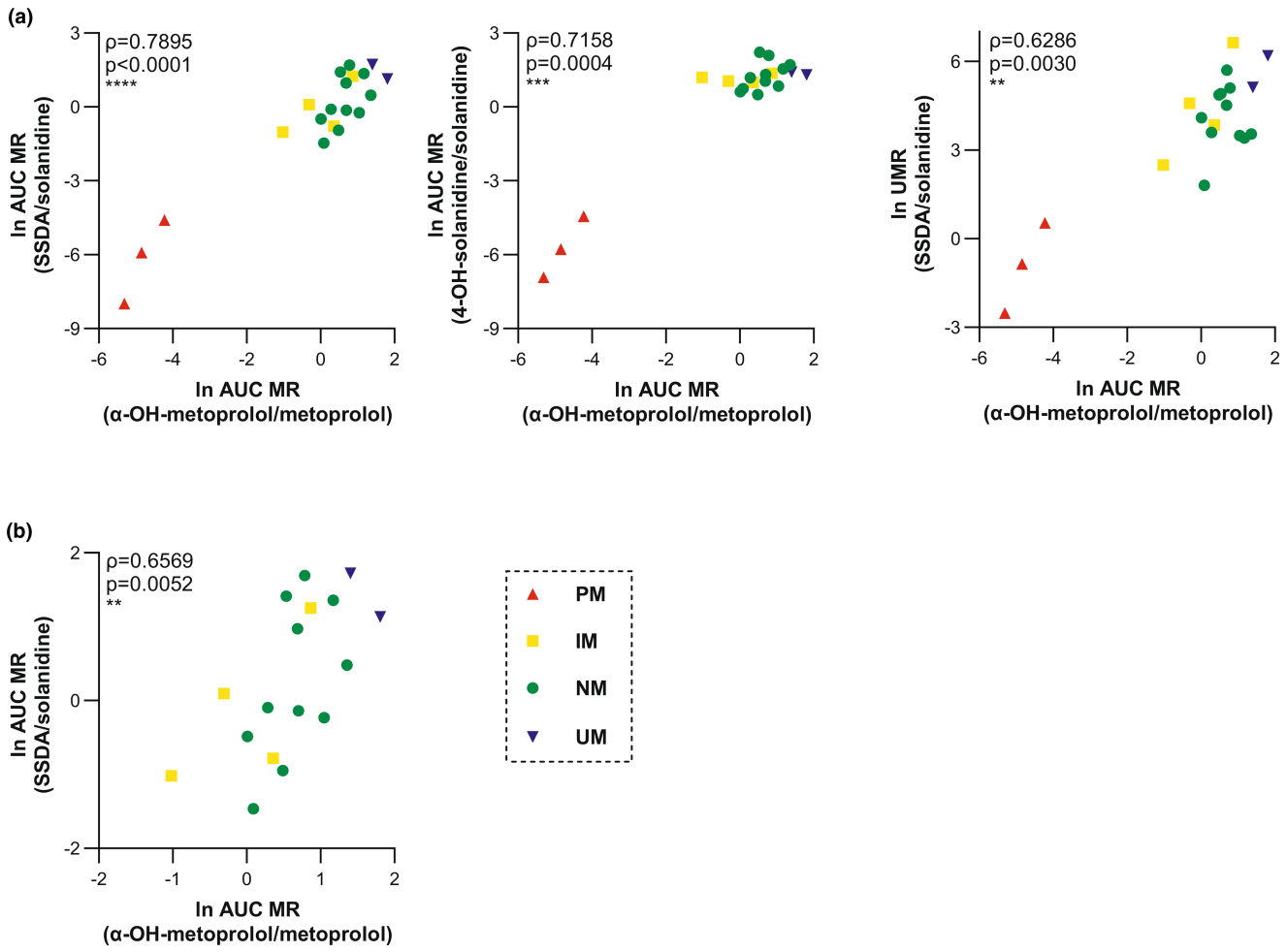

Figure 2 Correlation of solanidine AUC metabolic ratios to α-OH-metoprolol/metoprolol AUC_{0-8h} metabolic ratio. (**a**) Correlation of metabolite/solanidine MRs from plasma and urine to AUC MR α-OH-metoprolol/metoprolol. (**b**) Correlation of AUC MR SSDA/solanidine to MR α-OH-metoprolol/metoprolol without PM. Spearman correlation coefficient ρ and P value (2-tailed) are given. ** < 0.005, *** < 0.0005, **** < 0.0001. AUC, area under curve; IM, intermediate metabolizer; MR, metabolic ratio; NM, normal metabolizer; PM, poor metabolizer; SSDA, 3,4-secosolanidine-3,4-dioic acid; UM, ultrarapid metabolizer; UMR, urinary metabolic ratio.

α-OH-metoprolol/metoprolol with a spearman ρ of 0.7895 and a P value of < 0.0001 (**Figure 2a**). The correlation with partial AUC MR SSDA/solanidine is shown in **Table S1**. The correlation was still significant after excluding PM subjects, with Spearman ρ of 0.6569 and a P value of 0.0052 (**Figure 2b**). Due to the strong correlations of the AUC MR SSDA/solanidine, we tested if the MR SSDA/solanidine from single timepoint measurements still correlated with the AUC MR α-OH-metoprolol/metoprolol. Again, we observed a significant correlation at all timepoints (**Table 2**).

Due to its minimal invasiveness, we further addressed the solanidine and metabolite levels in a single urine sample of each subject. Solanidine, SSDA, or 4-OH-solanidine were detected in all urine samples. Urinary MR 4-OH-solanidine/solanidine did not show a significant correlation to AUC MR α-OH-metoprolol/metoprolol (data not shown). The correlation of urinary MR SSDA/solanidine to plasma AUC MR α-OH-metoprolol/metoprolol was significant with a P value of 0.003 and a Spearman ρ of 0.6286, but not as good as plasma MR SSDA/solanidine (**Figure 2a**).

The AUC MR SSDA/solanidine showed to correlate best with the metoprolol AUC MR; therefore, we assessed whether it could describe the genotype-predicted CYP2D6 phenotype. The AUC MR SSDA/solanidine and α-OH-metoprolol/metoprolol were compared with the CYP2D6 activity score in a linear regression analysis. CYP2D6 activity scores as predicted from the genetic diplotype were assigned according to a revised guideline from the CPIC.[17,18] Three PM subjects were not included in the analysis. Linear regression of both AUC MR SSDA/solanidine and AUC MR α-OH-metoprolol/metoprolol were significant with P values of 0.0330 and < 0.0001, respectively. The slope of the linear regression of the MR were 0.78 ± 0.33 and 0.80 ± 0.15 for SSDA/solanidine and α-OH-metoprolol/metoprolol, respectively (**Figure 3**).

DISCUSSION

In a recent CYP phenotyping cocktail study that was performed for another purpose, we addressed intraday variability of solanidine and its metabolites as CYP2D6 dietary biomarkers. We

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Table 2 Mean (±SD) log metabolic ratios for the respective genotype-predicted CYP2D6 phenotype and correlation with AUC MR α-OH-metoprolol/metoprolol

MR	Predicted CYP2D6 phenotype (N subjects)				Correlation to AUC α-OH-metoprolol/metoprolol (Spearman ρ, *P* value, significance)
	PM (3)	IM (4)	NM (11)	UM (2)	
AUC α-OH-metoprolol/metoprolol	−4.79±0.55	−0.02±0.82	0.65±0.43	1.61±0.28	
AUC SSDA/solanidine	−6.17±1.71	−0.11±1.03	0.23±1.03	1.43±0.28	0.7895; <0.0001; ****
AUC 4-OH-solanidine/solanidine	−5.71±1.24	1.15±0.17	1.26±0.58	1.36±0.08	0.7158; 0.0004; ***
Urinary SSDA/solanidine	−0.95±1.53	4.39±1.73	4.09±1.08	5.65±0.75	0.6286; 0.0030; **
TP 0 hour SSDA/solanidine	−6.05±1.83	−0.12±0.90	0.39±1.15	1.61±0.73	0.5956; 0.0133;* (without PM)
TP 2.5 hours SSDA/solanidine	−6.05±1.78	0.07±0.80	0.58±1.12	1.63±0.84	0.6863; 0.0031; ** (without PM)
TP 5 hours SSDA/solanidine	−6.17±1.74	0.03±0.82	0.31±1.32	1.65±0.77	0.6225; 0.0090; ** (without PM)
TP 8 hours SSDA/solanidine	−6.19±1.63	−0.13±0.92	0.10±1.08	1.65±0.72	0.6618; 0.0048; ** (without PM)
TP 24 h SSDA/solanidine	−6.19±1.76	−0.09±1.35	0.37±0.97	1.21±0.05	0.6225; 0.0090; ** (without PM)

Note: Spearman ρ, *P* value, and significance for correlation are shown. *<0.05, **<0.005, ***<0.0005, ****<0.0001.
Abbreviations: AUC, area under curve; IM, intermediate metabolizer; MR, metabolic ratio; NM, normal metabolizer; PM, poor metabolizer; SSDA, 3,4-secosolanidine-3,4-dioic acid; TP, timepoint; UM, ultrarapid metabolizer.

studied the association with the CYP2D6 phenotype determined by MR AUC α-OH-metoprolol/metoprolol.

The following key insights were gained from the study which are discussed in further detail in the following sections: (1) solanidine or its metabolites were detectable in plasma and urine of all study subjects, (2) SSDA was the most stable analyte over the day followed by 4-OH-solanidine and solanidine, (3) MR SSDA/solanidine correlated with the CYP2D6 phenotype at all measured timepoints, (4) MR SSDA/solanidine correlated better with the CYP2D6 phenotype than 4-OH-solanidine/solanidine, and (5) solanidine MR in plasma were better than urinary MR.

The dietary intake of solanidine was not known, yet we detected either solanidine or its metabolites in all 20 subjects. This is in accordance with prior studies from therapeutic drug monitoring (TDM) routine which revealed solanidine being abundant in human plasma samples, at least in the Northern or Central European regions.[14,15] The most common solanidine intake is probably from potatoes, but also tomatoes or eggplants could be a source of solanidine.[19] The abundance of solanidine in human plasma suggests that, in populations with a high dietary solanidine intake, no deliberate intake of solanidine containing food, in combination with a highly sensitive LC–MS method, is necessary to determine CYP2D6 activity. Although there is sparse information about kinetic parameters for solanidine and metabolites, they seem to have a slow elimination with long half-lives.[20,21] This was strengthened in a recent TDM study, which detected either solanidine or its metabolites in 97.8% of their tested samples.[15] In our study, we used a specific MRM method for the respective analytes, which may be even more sensitive than full-scan analyses from previous studies.[14,15] This highly sensitive method could explain that solanidine was detected in plasma of all subjects, as well as 4-OH-solanidine and SSDA in all non-PM subjects (**Figure 1**, **Table 1**).

Over the 5 sampling timepoints covering 24 hours, SSDA was the most stable analyte with a median SD of 11.62% of the individual subjects (**Table 1**). Solanidine and 4-OH-solanidine showed higher intraday variability with median SD of 21.66% and 13.20%, respectively (**Table 1**). This order of decreasing variability corresponds to the proposed metabolic pathway in which solanidine is metabolized by CYP2D6 to 4-OH-solanidine, which is further metabolized via intermediates to SSDA.[13] A potentially delayed formation might be the reason why SSDA is less prone to variation from dietary solanidine intake. In addition, SSDA might be a terminal metabolite in the solanidine metabolic pathway,[13] which could also contribute to its high stability over time. Solanidine metabolite levels alone cannot sufficiently depict the CYP2D6 phenotype without information about dietary intake. However, due to higher intraday variability of solanidine levels, probably influenced by dietary intake, the MR 4-OH-solanidine/solanidine and SSDA/solanidine showed higher intraday variability than the metabolites alone (**Table 1**). Pronounced changes in solanidine levels, but not SSDA, over time in some subjects may suggest a dietary intake of solanidine the day before or during the study, which may have affected the variability of the MR SSDA/solanidine.

In this cocktail study, the MR α-OH-metoprolol/metoprolol was used as a validated CYP2D6 phenotyping substrate.[6,22] Although the formation of α-OH-metoprolol is not entirely CYP2D6-exclusive, but with minor contributions of CYP3A4, CYP2B6, and CYP2C9,[22] metoprolol is recommended by the European Medicines Agency (EMA) as an *in vivo* CYP2D6 probe drug.[23] The AUC_{0-24h} MR SSDA/solanidine showed a strong correlation with AUC MR α-OH-metoprolol/metoprolol in plasma with a spearman ρ of 0.7895 (**Figure 1a**), which is stronger than correlations among validated CYP2D6 probe drugs, such as sparteine, debrisoquine, metoprolol, and dextromethorphan.[24] Correlations of MR SSDA/solanidine with MR α-OH-metoprolol/metoprolol in our study tended to be stronger compared with correlations with MR 9-hydroxyrisperidone/risperidone reported from analysis in TDM data,[15] which may be explained by the involvement of CYP3A4 in 9-hydroxyrisperidone formation.[25] Even all partial AUC MR SSDA/solanidine of 0–2.5, 0–5, and 0–8 hours showed similar strong correlations with spearman ρ above 0.7669 (**Table S1**). A subsequent correlation analysis of the single timepoint

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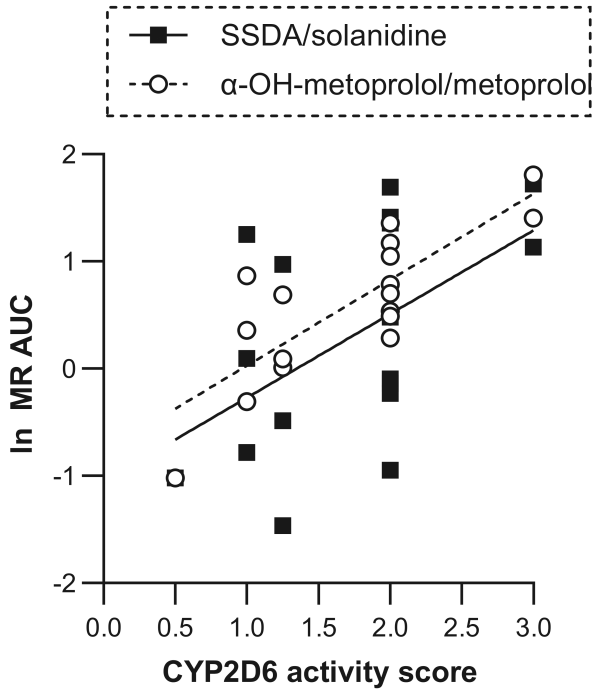

	SSDA/ solanidine	α-OH-metoprolol/ metoprolol
Slope ± SE	0.78 ± 0.33	0.80 ± 0.15
r^2	0.27	0.65
p-value	0.0330, *	<0.0001, ****

Figure 3 Metabolic ratios of SSDA/solanidine and α-OH-metoprolol/metoprolol in comparison to CYP2D6 activity score. PM subjects ($n=3$) were not included into the analysis. Linear regression analysis was performed with slope ± standard error, r^2 and P value given. * < 0.05, **** < 0.0001. AUC, area under curve; MR, metabolic ratio; PM, poor metabolizer; SSDA, 3,4-secosolanidine-3,4-dioic acid.

measurements of MR SSDA/solanidine to AUC MR α-OH-metoprolol/metoprolol showed significance at all timepoints even without PM subjects (**Table 2**), which suggests that not only the detection of PM subjects as described earlier,[14] but also phenotyping with MR SSDA/solanidine is possible with single timepoint measurements at any time throughout the day. This is even more striking as dietary intake of solanidine was uncontrolled in the study, resembling real-world conditions with variable intake of solanidine containing vegetables.

Interestingly, the correlation of MR 4-OH-solanidine/solanidine to MR α-OH-metoprolol/metoprolol was not as strong as MR SSDA/solanidine (**Figure 2a**). Similar results for MR 4-OH-solanidine/solanidine were reported by others, which was weaker in correlation to MR 9-hydroxyrisperidone/risperidone and dextrorphan/dextromethorphan, than MR SSDA/solanidine.[9,15] In one TDM study, MR 4-OH-solanidine/solanidine was claimed to be the best marker for the detection of PM subjects.[14] In general, however, 4-OH-solanidine MR might be less specific for assessment of CYP2D6 activity than SSDA, supported by the fact that 4-OH-solanidine was detected in 1 of 3 PM subjects (**Figure 1a**). In addition, the urinary MR SSDA/solanidine significantly correlated with AUC MR α-OH-metoprolol/metoprolol, but MR 4-OH-solanidine/solanidine in urine did not, although PM subjects could be detected by missing urinary 4-OH-solanidine levels in the presence of solanidine. Our results are similar to correlations described with dextrorphan/dextromethorphan, where solanidine MR in plasma correlated better than urinary MR.[9] However, even if less accurate in phenotype prediction, phenotyping from urinary MR would be more feasible in many occasions because it is even less invasive and easier to perform than in plasma.

This study has limitations, such as the smaller sample size of the study cohort compared with previously reported TDM studies.[14,15] However, compared with TDM studies our study was performed in highly standardized conditions with a defined dosage of metoprolol and sampling times, which allowed to calculate the MR from the AUC. Despite the limited cohort size, we had a broad representation of different genotype-predicted phenotypes with 3 PMs, 4 IMs, 11 NMs, and 2 UMs. Similar to previous studies, these genotyping results do not include rare variants, which might lead to erroneous assignments of activity scores. Our analyses were part of a phenotyping cocktail study, in which the precise dietary habits of the study participants prior and during the study were unknown; therefore, pharmacokinetic parameters, such as plasma half-lives of solanidine and metabolites, could not be assessed. In fact, standardization of solanidine dosing or exposure seems to be difficult due to multiple dietary sources. Future studies may further characterize variability in solanidine levels as well as kinetic parameters of solanidine and metabolites in correlation to specified food intake. Similar to previous studies, chemical standards and internal standards of solanidine metabolites were not available. In future applications, standards would allow to perform a more precise absolute quantification of the metabolites.

The AUC MR SSDA/solanidine and AUC MR α-OH-metoprolol/metoprolol correlated strongly and both showed a

Table 3 Phenotyping recommendations for solanidine and metabolites for different objectives

Objective	Phenotyping method; sample type	Rationale
Phenotyping in conditions where no blood samples are available	MR SSDA/solanidine; single urine sample	Less accurate, but minimal invasive
Phenotyping in clinical practice	MR SSDA/solanidine; single plasma sample	Accurate prediction possible
Drug–drug interaction studies, (inhibition, phenoconversion)	AUC MR SSDA/solanidine; repeated measurements in plasma	Most robust prediction, less prone to variation

Abbreviations: AUC, area under curve; MR, metabolic ratio; SSDA, 3,4-secosolanidine-3,4-dioic acid.

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significant linear relationship with the CYP2D6 activity score with a similar slope (**Figure 3**). This might suggest, that both MR are able to discriminate between CYP2D6 activity levels in a similar way. In fact, in a previous study, the MR SSDA/solanidine showed a significant decrease after inhibition with CYP2D6 inhibitor paroxetine.[9] For phenoconversion or drug–drug interaction studies, where multiple blood sampling may be available, we suggest to build the MR of the AUC to further increase the robustness and precision of the measurements (**Table 3**). The MR SSDA/solanidine might replace metoprolol or other CYP2D6 probe drugs in a cocktail study setting, allowing the reduction of administered drugs, thereby reducing the risk for adverse events and potential interactions between probe drugs.[8]

The MR SSDA/solanidine correlated with the AUC MR α-OH-metoprolol/metoprolol at all measured single timepoints, even after exclusion of PM subjects. This robust and reproducible correlation over the day clearly supports the use of single timepoint measurements for CYP2D6 phenotyping in a clinical setting. CYP2D6 phenotyping with solanidine might be specifically helpful in vulnerable patients, such as children, older adults, or polymedicated people, in which a probe drug administration might not be feasible (**Table 3**).

In conditions where no plasma samples are available, urinary MR SSDA/solanidine may be used for phenotyping (however less accurate).

In conclusion, the MR SSDA/solanidine correlated with the CYP2D6 phenotype (AUC MR α-OH-metoprolol/metoprolol) at each of the single timepoint measurements even after the exclusion of PMs, confirming the use of solanidine for CYP2D6 phenotyping in clinical settings where blood sampling is limited. In clinical studies with multiple blood sampling, the AUC MR SSDA/solanidine may be used to increase robustness and precision.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.P.M., J.S., and J.C.S. wrote the manuscript. J.P.M., P.Z., J.R., M.S., T.K., and J.C.S. designed the research. J.P.M., J.S., P.Z., R.T., J.R., E.S., and K.S.J. performed the research. J.P.M. analyzed the data. J.P.M. and J.S. contributed new analytical tools.

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