Article scientifique   Article   2016   Published version   Open Access

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Evaluation of Mutual Drug–Drug Interaction within Geneva Cocktail for Cytochrome P450 Phenotyping using Innovative Dried Blood Sampling Method

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Abstract: Cytochrome P450 (CYP) activity can be assessed using a 'cocktail' phenotyping approach. Recently, we have developed a cocktail (Geneva cocktail) which combines the use of low-dose probes with a low-invasiveness dried blood spots (DBS) sampling technique and a single analytical method for the phenotyping of six major CYP isoforms. We have previously demonstrated that modulation of CYP activity after pre-treatment with CYP inhibitors/inducer could be reliably predicted using Geneva cocktail. To further validate this cocktail, in this study, we have verified whether probe drugs contained in the latter cause mutual drug–drug interactions. In a randomized, four-way, Latin-square crossover study, 30 healthy volunteers received low-dose caffeine, flurbiprofen, omeprazole, dextromethorphan and midazolam (a previously validated combination with no mutual drug–drug interactions); fexofenadine alone; bupropion alone; or all seven drugs simultaneously (Geneva cocktail). Pharmacokinetic profiles of the probe drugs and their metabolites were determined in DBS samples using both conventional micropipette sampling and new microfluidic device allowing for self-sampling. The 90% confidence intervals for the geometric mean ratios of AUC metabolite/AUC probe for CYP probes administered alone or within Geneva cocktail fell within the 0.8–1.25 bioequivalence range indicating the absence of pharmacokinetic interaction. The same result was observed for the chosen phenotyping indices, that is metabolic ratios at 2 hr (CYP1A2, CYP3A) or 3 hr (CYP2B6, CYP2C9, CYP2C19, CYP2D6) post-cocktail administration. DBS sampling could successfully be performed using a new microfluidic device. In conclusion, Geneva cocktail combined with an innovative DBS sampling device can be used routinely as a test for simultaneous CYP phenotyping.

Human cytochromes P450 (CYP) represent one of the most important drug-metabolizing enzyme systems. The observed differences in their function are one of the major factors contributing to the interindividual variability of drug pharmacokinetics and thus of drug response. These differences are mainly due to diverse genetic polymorphisms as well as disease states, environmental influences or drug–drug interactions [1]. The real-time *in vivo* activity of CYPs (phenotyping) can be assessed using specific probe drugs.

Diverse phenotyping cocktails involving the simultaneous administration of a combination of probe drugs have been developed over the past two decades [2–6]. With the gain of scientific knowledge and the advances in analytical technologies, significant improvements in the cocktail approach have been observed in recent years. The authors of some of the recent cocktails have concentrated their efforts in developing phenotyping strategies using low probe dose [7,8] whereas others worked more on the aspects of alternative, less-invasive sampling methods [9,10].

In this optic, we have recently developed the Geneva phenotyping cocktail [11]. This cocktail represents an excellent combination of the use of low-dose probes with a low-invasiveness dried blood spots (DBS) sampling technique and a single sensitive analytical method for the phenotyping of six major CYP isoforms. CYP phenotyping combined with DBS approach has been proposed in another study which included different probes for CYP2B6, 2C9 and 2D6 at low but therapeutic doses. However, the sensitivity of the used method in the latter study was insufficient, and the concentration in the non-volumetrically applied DBS for most of the metabolites was below the limit of quantification [12].

Unlike other cocktails which are usually validated under 'normal' CYP function conditions, we have also demonstrated that the use of Geneva phenotyping cocktail combined with the DBS sampling technique could reliably predict modulation of CYP activity after pre-treatment with CYP inhibitors/inducer [11].

Besides showing that modulation in CYP activity can be accurately predicted, another important step in validating a cocktail is to verify that the combination of probe drugs can be used without mutual interactions [13]. It is in this aim that we conducted the latter study. In the goal of further simplifying sampling, we have also tested the precision and utility of a new microfluidic-based device for DBS sampling [14] in the context of the study. This easy-to-use device, allowing for precise volume collection, was shown to circumvent DBS-related issues such as haematocrit impact. As it can be used by untrained personnel, it has a great potential in providing a

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simple solution for sampling in various settings and for phenotyping in particular.

Materials and Methods

Subjects. After giving written informed consent, 30 healthy Caucasian volunteers (15 women, 15 men) were included in the study. The median age was 23.5 years (range, 18–36), and median BMI was 21.7 (range, 18.4–27.7). All of the subjects were non-smokers and had normal results on physical examination and liver function tests and were not taking any medications influencing CYP or P-gp function with the exception of hormonal contraception for eight women (six had oral contraception and two had hormonal implant). Urinary pregnancy tests were negative for all women at the inclusion and at the morning of each study session. The study sessions for women using oral contraception were scheduled outside of the 1-week monthly oral contraception break period. Poor CYP2D6 (*4/*4; *4/*5; *5/*5), CYP2C9 (*2/*2; *2/*3; *3/*3) or CYP2C19 (*2/*2) metabolizers were not included in the study. Subjects were not permitted to drink grapefruit juice for at least 1 week before and throughout the study and were required to abstain from alcohol and caffeine-containing products at least 48 hr before each study session.

This study (registration NCT02391688) was approved by the Ethics Committee of Geneva University Hospitals (ID: 14-061) and the Swiss Agency for Therapeutic Products (Swissmedic, Bern).

Study design. This study was an open-labelled, randomized, four-way, Latin-square, crossover study conducted at the Clinical Research Center of Geneva University Hospitals (Geneva, Switzerland). The four treatment arms were composed of A: caffeine 50 mg, flurbiprofen 10 mg, omeprazole 10 mg, dextromethorphan 10 mg and midazolam 1 mg; B: fexofenadine 25 mg; C: bupropion 20 mg; and D: the seven-drug cocktail regimen of caffeine 50 mg, bupropion 20 mg, flurbiprofen 10 mg, omeprazole 10 mg, dextromethorphan 10 mg, midazolam 1 mg and fexofenadine 25 mg (Geneva cocktail). Treatment A was composed of probe drugs with a documented lack of mutual interactions [4,15]. All of the treatments used in this study, with the exception of omeprazole 10 mg (Antramups® 10, AstraZeneca AG, Zug, Switzerland), were prepared as capsules using commercially available drugs and substances at the Pharmacy of Geneva University Hospitals under GMP conditions.

Based on probes and metabolites half-lives, a washout period of at least 1 week was respected between the sessions insuring a complete clearance of the drugs.

At each session, in the morning after an overnight fast, volunteers received orally with a glass of water one of the treatments A, B, C or D. Capillary blood samples from a small finger prick (BD Microtainer, Contact-Activated Lancet, Plymouth, UK) were collected before (time 0) as well as 0.5, 1, 2, 3, 4, 6 and 8 hr after drug administration. Two supplementary capillary blood samples 12 and 24 hr after drug administration were collected when treatment C or D was given (due to the longer half-life of OH-bupropion). Capillary whole blood (10 μl) was collected on a Whatman 903 filter paper card (St. Louis, MO, USA) using a volumetric micropipette (Rainin, Oakland, CA, USA). In parallel, capillary blood was also collected using a new collection device (HemaXist) integrating a patented microfluidic plate (WO/2013/144743) (DBS System, SA, Gland, Company, Switzerland) allowing for the accurate volume control (10 μl) and a conventional filter paper card (Perkin Elmer 226 Bioanalysis Card, Greenville, SC, USA) for blood storage [14].

The blood drop was applied at the entrance of the microfluidic channel. Once filled, the cover containing filter paper card (Perkin Elmer 226 Bioanalysis Card) was folded transferring the controlled blood volume (10 μl) onto the card.

After a 30-min. drying period at room temperature, DBS cards were packed in sealable plastic bags and stored at −20°C.

Analytical methods. The cocktail substrates and their CYP-specific metabolites have been quantified in DBS using a single reversed-phase high-performance liquid chromatography–tandem mass spectrometry method (HPLC-MS/MS) operating in dual electrospray ionization mode, as previously described [16]. Briefly, discs of 6 mm diameter covering the entire DBS were punched out and folded into the bottom of individual LC vials containing a 300-μl inert insert. For the extraction, methanol (100 μl) containing the internal standards was added to each vial. The vials were then sealed, vortex-mixed and positioned in the LC rack.

Dried blood spots analysis was performed using a LC–MS/MS system consisting of a 5500QTrap® triple quadrupole linear ion trap (QqQ$_{LIT}$) mass spectrometer (AB Sciex, Toronto, ON, Canada) and an Ultimate 3000 RS instrument (Dionex, Sunnyvale, CA, USA) as LC system. The chromatographic separation was conducted on a Kinetex™ RP C18 XB column (2.1 × 50 mm, 2.6 μm; Phenomenex, Torrance, CA, USA) with water and acetonitrile as mobile phase at a flow rate of 0.6 ml/min. A linear gradient was employed from H$_2$O/ACN (98/2, v/v) to H$_2$O/ACN (2/98, v/v) over 2 min. The used method was fully validated according to international criteria with intermediate precision values of less than 10.9% and accuracy in the interval 92.2–111.1% for all substances and all concentrations tested [16].

Genotyping. Genomic DNA was extracted from whole blood (200 μl) using the QIAamp DNA blood mini kit (QIAGEN, Hombrechtikon, Switzerland). The detection of *CYP2C19* (alleles *2 and *3) and 33 *CYP2D6* alleles was performed using the AmpliChip CYP450 test (Roche Diagnostics, Basel, Switzerland) as previously described [17]. *CYP2B6*6* and *CYP2C19*17* genotypes were determined using commercially available TaqMan® SNP genotyping assays. *CYP2C9*2* and *CYP2C9*3* genotypes were determined by means of multiplex PCR with fluorescent probes (Lightmix, TIB Molbiol, Berlin, Germany) and melting curve analysis on a LightCycler480 (Roche Diagnostics).

Statistical analysis. The intrasubject coefficients of variation (CVs) for the AUC ratios of the administered cocktail probe drugs vary between 8% and 30% [4,6,11,18]. On the basis of the highest CV (30%), the sample size for a crossover design study with an acceptance range of 0.8–1.25 for the confidence interval and a μT/μR value of 1.0 (i.e. absence of interaction) was estimated to be 30 (power of 80% and α value of 5%).

Pharmacokinetic parameters were estimated by standard non-compartmental methods using WinNonlin version 6.2.1 (Pharsight, Mountain View, CA, USA). AUC ratios were determined as the ratio between the AUC of the metabolite and the AUC of the administered probe. Single-point metabolic ratios (MRs) were determined as the concentration ratio between the metabolite and the administered substance, that is paraxanthine/caffeine (par/caf), 4′-hydroxybupropion/bupropion (OHbup/bup), 4′-hydroxyflurbiprofen/flurbiprofen (OHflb/flb), 5′-hydroxyomeprazole/omeprazole (OHopz/opz), dextrorphan/dextromethorphan (dor/dem) and 1′-hydroxymidazolam/midazolam (OHmdz/mdz) at a given time-point. Results are presented as geometric mean values together with the geometric mean ratio and 90% confidence interval (CI). Geometric mean ratio for the AUC ratios or MRs was calculated as the ratio of the geometric mean obtained after administration of the probe alone (or as part of a validated five-drug cocktail) to that obtained after administration of the probe as part of the seven-drug cocktail. Absence of pharmacokinetic interaction could be claimed if the 90% confidence interval (CI) for the geometric mean ratio fell completely inside the 0.8–1.25 interval. Correlation between AUC ratios and single-point metabolic ratios was established using Spearman's rank correlation coefficient. The AUC ratios of

volunteers in different genotype groups were compared using a nonparametric Mann–Whitney *U*-test. A probability of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software version 22 (Chicago, IL, USA).

Results

Thirty volunteers participated in the study and successfully completed the four study sessions. One volunteer was excluded from analysis because two key samples were missing (2 and 3-hr sampling points at treatment A). None of the subjects reported side effects after single drug or cocktail administration.

Table 1 shows the geometric means, and their ratios, for the $AUC_{metabolite}/AUC_{probe}$ of probes administered alone (or as part of a previously validated cocktail) and as part of the Geneva phenotyping cocktail. The 90% confidence intervals (CIs) for the geometric mean ratios fell entirely within the 0.8–1.25 bioequivalence range indicating the absence of pharmacokinetic interaction.

In a previous study, we have determined the best limited-sampling phenotyping indices based on their correlation with AUC ratios [11]. These indices include single-point metabolic ratio (MR) at 2 hr for CYP1A2 and CYP3A, at 3 hr for CYP2B6, CYP2C9 and CYP2D6 and limited-sampling $AUC_{2,3,6}$ ratio for CYP2C19. The excellent correlation ($\rho_s \geq 0.918$, $p < 0.001$) between these phenotyping indices and AUC ratios has been confirmed in the current study (table 2). For CYP2C19, OHopz/opz MR at 3 hr, a phenotyping index simpler than $AUC_{2,3,6}$ ratio, was also highly correlated to OHopz/opz AUC ratio ($\rho_s = 0.875$, $p < 0.001$).

The 90% CIs for the geometric mean ratios of the selected phenotyping indices for each CYP were also included within the 0.8–1.25 range (table 3).

Individual comparison of the phenotyping indices between the sessions is shown in fig. 1. This figure also shows the gender and genotype distribution of metabolic ratios. Poor CYP2C9, CYP2C19 and CYP2D6 metabolizers were not included in the study (see Methods). One volunteer was identified as CYP2D6 intermediate metabolizer (CYP2D6*5/*10), and 14 volunteers were heterozygous with one loss-of-function (*3; *4 or *5) and one normal function alleles. Their MRs and AUC ratios differed significantly from the remaining homozygous carriers of normal function alleles ($p = 0.002$).

Table 2.

Spearman rank correlation (ρ_s) between AUC last ratios of metabolite/substance and the chosen phenotyping indices.

CYP	Phenotyping index	ρ_s	*p*-Value
1A2	[par]/[caf] at 2 hr	0.984	<0.001
2B6	[OHbup]/[bup] at 3 hr	0.938	<0.001
2C9	[OHflb]/[flb] at 3 hr	0.966	<0.001
2C19	$AUC_{2,3,6\ OHopz}/AUC_{2,3,6\ opz}$	0.918	<0.001
	[OHopz]/[opz] at 3 hr	0.875	<0.001
2D6	[dor]/[dem] at 3 hr	0.994	<0.001
3A	[OHmdz]/[mdz] at 2 hr	0.920	<0.001

AUC last, AUC from 0 to 8 hr, except for OHbup and bup, where AUC last = AUC from 0 to 24 hr. AUC2,3,6, AUC calculated based on concentrations at three-sampling points (2, 3 and 6 hr post-drug administration); AUC, area under the concentration–time curve; bup, bupropion; caf, caffeine; CYP, cytocromeP450; dem, dextromethorphan; dor, dextrorphan; mdz, midazolam; OHbup, 4′-hydroxybupropion; OHflb, 4′-hydroxyflurbiprofen; OHmdz, 1′-hydroxymidazolam; OHopz, 5′-hydroxyomeprazole; opz, omeprazole; par, paraxanthine.

Similarly, carriers of reduced function CYP2C9 (CYP2C9*1/*2, n = 7; CYP2C9*1/*3, n = 1) and CYP2B6 (CYP2B6*1/*6, n = 16; CYP2B6*6/*6, n = 1) alleles had significantly lower AUC and metabolic ratios in comparison with wild types ($p < 0.04$). Statistically, heterozygous loss-of-function CYP2C19*2 carriers (n = 7) had significantly lower MRs in comparison with wild-type (n = 11) volunteers ($p = 0.013$), whereas carriers of the enhanced function allele CYP2C19*17 (10 heterozygous, one homozygous) did not differ from wild-type ($p = 0.18$). Female volunteers had lower OHopz/opz metabolic ratio ($p = 0.012$). Gender difference was statistically significant also for CYP1A2 function ($p = 0.03$). However, the difference was no longer significant when men were compared only with women without hormonal contraception ($p = 0.36$).

Fexofenadine, which was administered as part of the cocktail and as a probe for p-glycoprotein, did not have an impact on the pharmacokinetics and AUC ratios of CYP probe drugs. Detailed results on the impact of CYP probe drugs on fexofenadine disposition as well as several issues regarding its use as a P-gp probe will be presented in a separate article.

Concentrations obtained with the microfluidic sampling device showed excellent correlation with conventional micropipetting concentrations with slopes values close to 1 (0.91–1.03) and determination coefficients $R^2 > 0.90$ for all of

Table 1.

Geometric means and 90% CI for the AUC metabolite/probe ratios.

CYP	Parameter (AUC ratio)	Geometric mean			90% CI for the ratio	
		Probe alone or five-drug cocktail	Geneva cocktail	Ratio	Lower limit	Upper limit
1A2	$AUC_{0-8\ hr}$ paraxanthine/$AUC_{0-8\ hr}$ caffeine	0.39	0.41	0.97	0.88	1.08
2B6	$AUC_{0-24\ hr}$ OH-bupropion/$AUC_{0-24\ hr}$ bupropion	4.61	4.98	0.93	0.84	1.03
2C9	$AUC_{0-8\ hr}$ OH-flurbiprofen/$AUC_{0-8\ hr}$ flurbiprofen	0.041	0.043	0.95	0.87	1.04
2C19	$AUC_{0-8\ hr}$ OH-omeprazole/$AUC_{0-8\ hr}$ omeprazole	0.75	0.66	1.13	1.04	1.22
2D6	$AUC_{0-8\ hr}$ dextrorphan/$AUC_{0-8\ hr}$ dextromethorphan	1.82	1.73	1.05	0.91	1.21
3A	$AUC_{0-8\ hr}$ OH-midazolam/$AUC_{0-8\ hr}$ midazolam	0.42	0.48	0.88	0.80	0.96

AUC, area under the concentration–time curve; CI, confidence interval; CYP, cytochrome P450.

Table 3.

Geometric means and 90% CI for the chosen phenotyping index.

CYP	Phenotyping index	Geometric mean			90% CI for the ratio	
		Probe alone or five-drug cocktail	Geneva cocktail	Ratio	Lower limit	Upper limit
1A2	[par]/[caf] at 2 hr	0.29	0.31	0.95	0.84	1.07
2B6	[OHbup]/[bup] at 3 hr	2.51	2.73	0.92	0.82	1.03
2C9	[Ohflb]/[flb] at 3 hr	0.043	0.046	0.93	0.86	1.01
2C19	$AUC_{2,3,6\ OHopz}/AUC_{2,3,6\ opz}$	0.84	0.79	1.05	0.92	1.20
	[OHopz]/[opz]at 3 hr	1.04	0.99	1.05	0.90	1.23
2D6	[dor]/[dem] at 3 hr	1.70	1.63	1.04	0.90	1.19
3A	[OHmdz]/[mdz] at 2 hr	0.44	0.50	0.87	0.80	0.96

$AUC_{2,3,6}$, AUC calculated based on concentrations at three-sampling points (2, 3 and 6 hr post-drug administration); AUC, area under the concentration–time curve; bup, bupropion; caf, caffeine; CI, confidence interval; CYP, cytocromeP450; dem, dextromethorphan; dor, dextrorphan; mdz, midazolam; OHbup, 4′-hydroxybupropion; OHflb, 4′-hydroxyflurbiprofen; OHmdz, 1′-hydroxymidazolam; OHopz, 5′-hydroxyomeprazole; opz, omeprazole; par, paraxanthine.
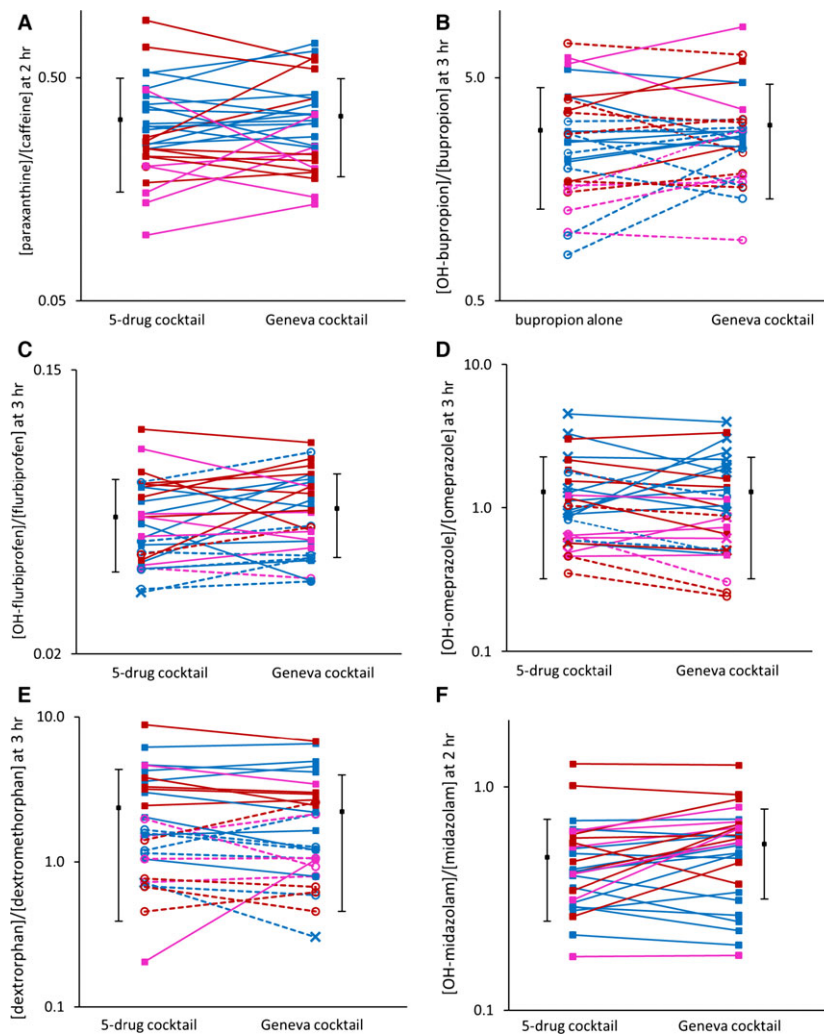

Fig. 1. Individual comparison of phenotyping indices after administration of probe alone (or as part of a validated five-drug cocktail) *versus* Geneva cocktail. Blue line indicates a male volunteer, pink line indicates a female volunteer receiving hormonal contraception, and red line indicates female volunteer without hormonal contraception. (B) Dashed line (open circles) = CYP2B6*1/*6 and *6/*6 carriers. (C) Dashed line (cross) = CYP2C9*1/*3 carrier, dashed line (open circles) = CYP2C9*1/*2 carriers. (D) Dashed line (open circles) = CYP2C19*1/*2, continuous line (cross) = CYP2C19*1/*17 carriers. (E) Dashed line (cross) = 2D6 intermediate metabolizer (*5/*10); dashed line (open circle) = CYP2D6*1/*4; *2/*4 and *2/*5 carriers.
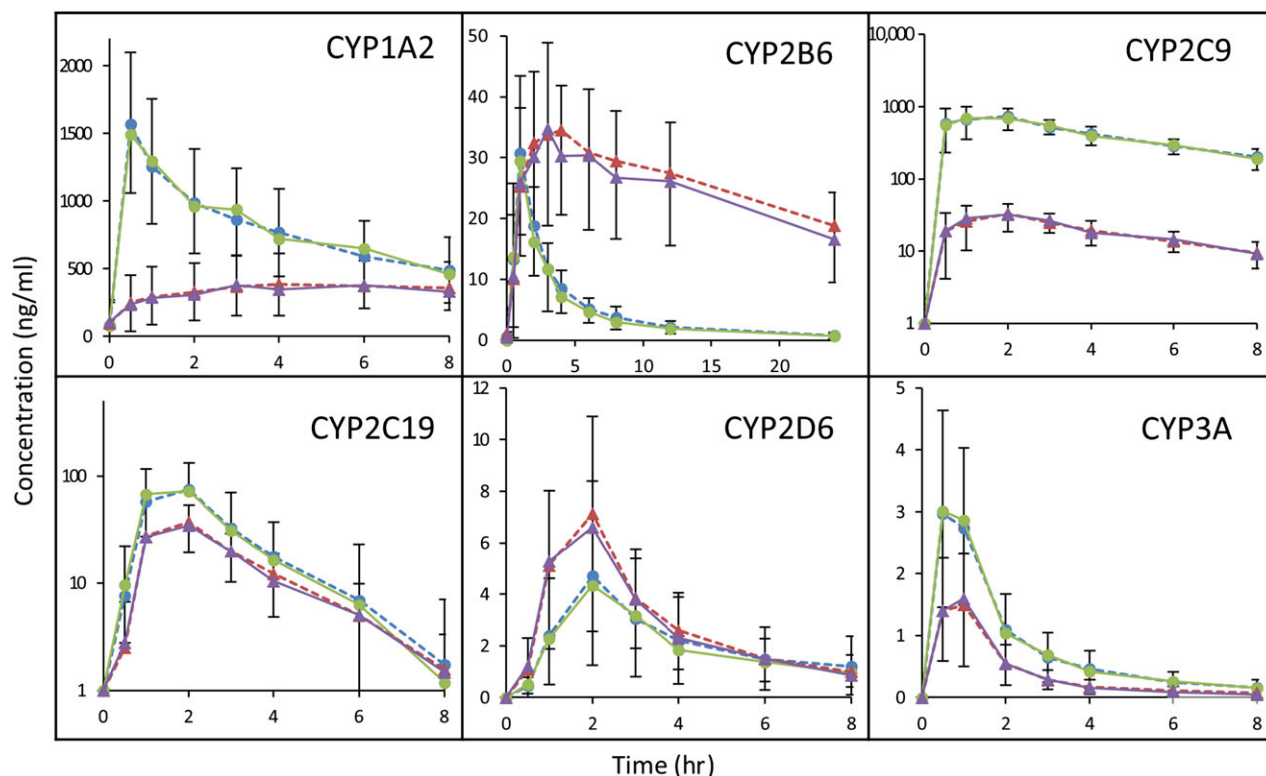
Fig. 2. Concentration–time profiles for CYP probe substrates (circles) and their metabolites (triangles) in 10-μl capillary DBS samples obtained using volumetric pipette (dashed lines) or microfluidic device(continuous lines) after oral administration of Geneva cocktail in 29 healthy volunteers. (A) Caffeine and paraxanthine (CYP1A2), (B) bupropion and OH-bupropion (CYP2B6), (C) flurbiprofen and OH-flurbiprofen (CYP2C9), (D) omeprazole and OH-omeprazole (CYP2C19), (E) dextromethorphan and dextrorphan (CYP2D6), (F) midazolam and OH-midazolam (CYP3A). Error bars represent standard deviation.

the substances. As a consequence, the pharmacokinetic profiles for CYP probes and their metabolites administered as part of the cocktail were almost identical with both sampling methods (fig. 2).

Discussion

In this study, we have evaluated the interaction potential between probe drugs contained within the Geneva phenotyping cocktail. This cocktail composed of widely available and safe low-dose probes was investigated in a previous study and was shown to reliably predict the modulation of CYP activities after inhibitor/inducer pre-treatment [11]. Unlike many other cocktails which use invasive or more than one sampling matrices [4,5,19], a single matrix (whole capillary blood) with a 10 μl DBS sampling technique is used with this cocktail. The use of this simple and low-invasiveness approach was previously validated, and probe and metabolite concentrations measured in capillary DBS were highly correlated with plasma concentrations [11,16].

In the current study, we have shown that there is no interaction between CYP probe drugs contained within the Geneva cocktail since the 90% CIs for the $AUC_{metabolite}/AUC_{probe}$ geometric mean ratios fell entirely within the 0.8–1.25 range for each enzyme (table 1). The lack of interaction between caffeine, flurbiprofen, omeprazole, dextromethorphan and

midazolam has been previously demonstrated [4,15,20]. The addition of a probe for CYP2B6 is an important advance since this enzyme is implicated in the metabolism of numerous clinically used drugs such as antineoplastics cyclophosphamide and ifosfamide, antiretrovirals nevirapine and efavirenz, anaesthetics propofol and ketamine, antidepressant bupropion, synthetic opioid methadone and few others [21]. Based on individual phenotyping studies [22] as well as on EMA [23] and FDA [24] recommendations, bupropion has been selected as CYP2B6 phenotyping drug. Few studies have shown that bupropion at steady-state (150 mg BID during more than 2 weeks) can inhibit CYP2D6 [25,26]. However, since it is considered that CYP2D6 inhibition is mainly mediated by bupropion's metabolites threohydrobupropion and erythrohydrobupropion which accumulate after multiple administrations, a single bupropion dose as low as 20 mg is not expected to have an inhibitory effect on CYP2D6 [11]. The lack of inhibitory effect of bupropion 20 mg on CYP2D6 has been confirmed with the results of the current study which show that the 90% CI for $AUC_{dextrorphan}/AUC_{dextromethorphan}$ was between 0.91 and 1.21 when dextromethorphan was administered without or with bupropion.

Whereas complete determination of probe and metabolite AUC, considered as gold standard, is feasible in the controlled setting of clinical studies, the multiple sampling that it requires makes it difficult to implement if the cocktail approach is to

be used in clinical practice. Therefore, limited-sampling strategies and simplified phenotyping indices such as single-point plasma MRs or urinary metabolic ratios have been proposed in several cocktail studies [4,12]. To reliably predict CYP function, the proposed phenotyping metrics need to fulfil several criteria such as good correlation with the partial clearance for the specific metabolic pathway, correlation with other validated metrics, reflection of known genetic polymorphisms or changes in metric when patients are treated with inhibitors/inducers of the enzyme [13]. Based on these criteria, in a previous study, we have determined the MRs at 2 hr for CYP1A2 and CYP3A, at 3 hr for CYP2B6, CYP2C9 and CYP2D6 and limited-sampling $AUC_{2,3,6}$ ratio for CYP2C19 as the best phenotyping indices both at normal and altered CYP function [11]. These indices, including an even more simplified CYP2C19 index (MR at 3 hr), showed great correlation with AUC ratios also in the current study. The lack of interaction between probe drugs was also confirmed using the phenotyping indices instead of AUC ratios as criteria.

As expected, heterozygous volunteers with diminished function alleles for CYP2B6, 2C9, 2C19 and 2D6 had generally lower metabolic ratios in comparison with homozygous volunteers with wild-type alleles.

Most of the previously published cocktails were evaluated only in men. This can be an issue because sex-specific side effects have been observed in a previous cocktail study [19]. In our study, the administered cocktail was well tolerated both by male and by female volunteers. No apparent differences were observed in the phenotyping indices for CYP2B6, 2C9 and 2D6 between man and women. It has been previously suggested that women might have a higher clearance of CYP3A substrates in comparison with men [27]. In our study, the $AUC_{OH\text{-midazolam}}/AUC_{midazolam}$ was slightly higher in women but did not reach statistical significance (0.59 ± 0.23 versus 0.45 ± 0.16). The apparent activity of CYP2C19 appeared to be lower in women. This difference could be partially explained by a higher proportion of CYP2C9*1/*2 and a lower proportion of CYP2C9*1/*17 carriers among women, and partially by the inhibition effect of hormonal contraception [28]. Similarly, CYP1A2 had lower apparent function in women, an observation which could also be potentially explained by the inhibitory effect of hormonal contraception [29]. The inhibitory effect of contraceptive pills on CYP2C19 and CYP1A2 activities has also been observed in a previous cocktail study [30].

Recently, De Kesel et al. [31] have reviewed the use of alternative sampling strategies such as DBS, oral fluid or exhaled breath for CYP phenotyping. They have concluded that DBS sampling is the preferred method based on criteria such as patient's comfort, sampling in the absence of specialized staff, ease of transport and storage, home-sampling potential and analysis using standard analytical equipment. The home-sampling potential and sampling in the absence of specialized staff of DBS were classified as very suitable. However, in order to obtain accurate quantitative data and to overcome the impact that haematocrit might have on the spreading of the blood spot, it is important to apply precise blood volume and analyse the whole blood spot. This can be carried out using a volumetric micropipette which is straightforward in an equipped laboratory with trained personnel. However, this method may be challenging when used within the course of clinical studies, in private practice or at home in the case of unsupervised sampling. To simplify this process, new precise volume sampling techniques have been developed [14,32]. One of these techniques involving the use of a simple microfluidic-based device incorporating 10 µl channels and commercially available DBS cards has been tested in this study [14]. Probe and metabolite concentrations obtained using this sampling method coincided with those obtained using a volumetric micropipette. Therefore, this device can replace micropipette sampling and can be used in combination with the Geneva phenotyping cocktail in clinical studies, hospitals and private practice settings.

In conclusion, in this study, we have demonstrated that the CYP probes contained within Geneva cocktail do not cause mutual interactions. This provides even more extensive validation of the cocktail which was previously shown to reliably predict both normal and altered CYP activity. The combination of this low-dose, high-throughput cocktail with a simple user-friendly capillary sampling device allows for its use as a phenotyping tool in various settings including hospitals, private practice or for studies in countries with limited resources.

Acknowledgements

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