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Safety and pharmacokinetics of the CIME cocktail and its metabolites after a single oral dosing in healthy volunteers

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Abstract

We designed the CIME cocktail (acetaminophen, caffeine, dextromethorphan, digoxin, memantine, midazolam, omeprazole, repaglinide, rosuvastatin and tolbutamide) to simultaneously assess the activities of six CYP (1A2, 2C8, 2C9, 2C19, 2D6, 3A4), one phase II enzyme (UDP-glucuronosyltransferase), two transporters (P-gp, OATP1B1) and renal tubular active reabsorption/secretion. We conducted a prospective clinical study in ten healthy volunteers to i) evaluate the CIME cocktail safety and ii) describe the pharmacokinetics of the ten probes and their metabolites following an oral, single-dose, simultaneous administration. We demonstrated that i) safety was good with only reversible mild or moderate side effects, (ii) the developed and validated analytical method was able to quantify simultaneously the 10 probe and the 7 major metabolite concentrations from 0.5 ml of blood and iii) it was possible to estimate by non-compartmental analysis pharmacokinetic parameters and phenotypic indexes.

1. Introduction

Therapeutic responses are subject to large intra- and inter-patient pharmacokinetic/pharmacodynamic variability. Pharmacokinetics includes absorption, metabolism and elimination rates dependent on enzyme activities and transport functions. The metabolites resulting from phase I metabolism are combined with hydrophilic moieties (phase II metabolism) and are actively eliminated through phase III efflux transporters. Such transporters are also active in eliminating some of the substrate or phase I metabolites. Expression of CYP and of phase II and III enzymes and also their activities depend on genetic as well as environmental factors and vary according to age, sex and health (1). This results in a wide degree of inter- and intra-individual variability in the rate and extent of drug metabolism and pharmacokinetics (DMPK)(2).

Several strategies have been developed to determine the metabolizer status of each individual included in a phase I clinical study or before starting a treatment involving drugs with a small therapeutic range, so as to maximize efficacy and minimize side effects (*i.e.* personalized treatment). Among them, the “cocktail strategy” initiated by Breimer and Schellens (3) consists in the simultaneous phenotyping of several CYP using a mixture of selective probes (*e.g.* midazolam for CYP3A4 and dextrometorphan for CYP2D6). The cocktail approach has been used in order to determine essentially the main hepatic CYP activities in various situations. These include the Pittsburgh cocktail for phenotyping of CYP 1A2, 2E1, 2C19, 2D6, 3A4 and N-acetyl transferase2 (NAT2) (4) and the Cooperstown or Cooperstown 5+1 cocktail for CYP 1A2, 2C9, 2C19, 2D6, 3A4, NAT2 and xanthine oxidase (XO) (5). A review of the requirements for appropriate phenotyping procedures has been published recently (6). Whereas these cocktails address the main liver CYP, few of them assess phase II enzymes activities (except NAT2 (4, 5) or NAT2 and XO (5, 7)) or transporters functions (except P-glycoprotein (8-10)). Recent advances in phase II enzymes knowledge, especially uridyl glucuronyltransferase (UGT), and transporters make possible the use of specific and efficient substrates as probes (11). Moreover, many of these previously developed cocktails associate both oral and *i.v.* route dosing (9), and require both plasma and urine collections and/or need prolonged collection times (5).

We recently undertook the development of the CIME (Metabolic Identify Card) cocktail. This cocktail possesses the interest of being made for phenotyping the main CYPs but also a phase II enzyme (UGT1A), two transporters (P-glycoprotein and OATP1B1) and renal active secretion and reabsorption. After development and validation of a simultaneous LC-MS/MS bioanalytical assay for the substrates of the CIME cocktail and main

relevant metabolites (12), this cocktail was used to characterize a blood-brain barrier model (13), to demonstrate the drug-metabolizing efficacy of a microfluidic biochip containing primary human hepatocyte cultures (14), and to phenotype several CYP activities in rat (15). Here, we conducted a prospective clinical study in healthy volunteers with the main objectives of i) evaluating the safety of the CIME cocktail given at doses allowing sufficient circulating levels for accurate bioanalytical determination, and ii) assessing the pharmacokinetic (PK) parameters of the CIME cocktail probes administered concomitantly, and their metabolites.

2. Results

Volunteers

A total of 10 healthy Caucasian subjects (6 males, 4 females) were enrolled in the study. Their mean age was 31.5 ± 7.7 years (range 19-42 years) and the mean body mass index was $23.3 \pm 3.6 \text{ kg.m}^{-2}$ (range 19.9-29.7).

The CYP2D6 genotypes were the following: 5 wild-type genotypes (subjects 1, 6, 7, 9, 10), 4 heterozygous genotypes for deficient alleles (*3, *4 or *6 alleles; subjects 2, 3, 5, 8) and 1 heterozygous composite genotype (*4/*5, subject 4). However, according to the high number of CYP2D6 polymorphisms (<http://www.cypalleles.ki.se/cyp2d6.htm>), we cannot exclude the presence of rare mutations not analyzed in this study in the subjects designated as having the wild-type genotype. Subject 4 with the two mutated alleles was classified as a poor metabolizer based on his genotype; the others were classified as extensive metabolizers. In the sequel, PK parameters and phenotypic indexes will be given separately for extensive and poor metabolizers. Several polymorphisms were also detected in the gene coding for CYP2C19 (*1/*1, n=5; *1/*17, n= 4; and *2/*17, n = 1) but all the volunteers were predicted as normal or ultrarapid 2C19 metabolizers.

Safety of the CIME cocktail

Nine out of ten healthy volunteers experienced adverse events: somnolence (n= 8 volunteers, apparition time = 40min-6h post-administration, duration = 20min – 3h) as expected after midazolam administration, dizziness

(n=3, apparition time = 40min-12h post-administration, duration = 5min-2h), headache (n=2, apparition time = 1h & 1 day post-administration, duration = 1h & less than 1 day), blood pressure drop (n=2, apparition time = 3h and 8h post-administration, duration = 3h and less than 1 day), nausea (n=1, apparition at 14h post-administration, duration = 30 min), atrial extrasystole (n=1, apparition time = 8h30 post-administration, disappeared at the day 1 follow-up visit), diarrhea (n=1, apparition time = 3h post-administration, duration = 30min) and abdominal pain (n=1, apparition time = 48h post-administration, duration = 1 day). All of these events were mild or moderate in severity. No serious adverse events were reported during the trial.

Table 1 summarizes parameters of the cardiovascular system, blood, and liver and kidney function. A non-parametric Wilcoxon signed rank test showed a small decrease between baseline and end-point values in red blood cell count (p-value=0.018), hemoglobin (p-value=0.016), hematocrit (p-value=0.014) and in the sitting diastolic blood pressure (p-value=0.044). However, all the corresponding values remained within the clinical range corresponding to healthy individuals.

ECG records revealed no abnormalities in any treated volunteers and were within normal limits (QT/QTc interval <450 ms for all the volunteers and at all the time-points). No hypoglycemia was noted. The range of observed digoxin maximum concentration (C_{max}) was 0.25-0.85 ng/ml. It remained in the therapeutic range (0.5- 1 ng/ml) and below the toxicity threshold (2.5 ng/ml) (16) for all the volunteers.

Pharmacokinetics

A total of 190 plasma samples were analyzed by LC-MS/MS. For two minor metabolites (3-methoxymorphinan and 4-hydroxy-midazolam), only few concentrations were above the low limit of quantification (LLQC): 41 samples from 6 volunteers for 3-methoxy-morphinan and 27 from 8 volunteers for 4-hydroxy-midazolam. Therefore no PK parameters were estimated for these two metabolites. For the 10 substrates and the 7 remaining metabolites, at least 6 consecutive samples with concentration above the LLQC were available per volunteer: a total of 166 (acetaminophen), 160 (acetaminophen glucuronide), 165 (caffeine), 165 (paraxanthine), 136 (dextromethorphan), 160 (dextrorphan), 184 (digoxin), 187 (memantine), 148 (midazolam), 166 (1-hydroxy-midazolam), 106 (omeprazole), 131 (5-hydroxy-omeprazole), 133 (omeprazole sulfone), 112 (repaglinide), 177 (tolbutamide) and 168 (4-hydroxy-tolbutamide) plasma concentrations obtained from 10

subjects were included in the PK analysis. The excluded samples corresponded to early or terminal sampling times (concentration <LLQC or re-intake of caffeine).

Individual plasma concentration time-courses are shown on Figures 1A, 1B and 1C (uncorrected data for caffeine and paraxanthine). As expected, the plasma concentration-time curve shapes of omeprazole and its metabolites were highly variable between individuals, notably in the absorption phase with variable lag-time, due the enteric-coated dosage form of omeprazole. In accordance with CYP2D6 genotype, subject 4 (classified as poor metabolizer for CYP2D6) presented high dextromethorphan concentration and low dextrorphan concentration compared to the other volunteers. Another outlier (considered as very different from the 8 other extensive metabolizers) was also identified for dextromethorphan: subject 9 (wild-type genotype for CYP2D6) with intermediate dextromethorphan concentrations and normal concentrations of dextrorphan.

Estimated PK parameters are summarized in Table 2. They are consistent with published studies where drugs were administrated alone (see supplementary information 1 and reference therein).

Phenotype assessment and comparison with genotype

Table 3 summarizes the phenotypic indexes for the 10 targets of the CIME cocktail. Inter-individual variability in the activity of CYP and transporters was apparent. As expected, quite high variability was observed for CYP3A4 phenotype (CV 83%). High difference was also observed for CYP 2D6 between the poor and extensive metabolizers. Indeed, PM subject 4 presents an AUC dextromethorphan/AUC dextrorphan MR of 80.9 vs 0.05-1.49 (range) for the 9 extensive metabolizers. The remaining subject (subject 9, no mutation) presented a quite high MR for extensive metabolizer (MR = 1.5) resulting of his unexpected high level of dextromethorphan. None of the 10 subjects was considered as PM from their genotype or phenotype for CYP2C19.

3. Discussion

Novelty of the CIME cocktail

Since the description of the first cocktail by Breimer et Schellens (3), several other cocktails have been developed for assessing the major CYP activities, some phase II enzymes, and for a few of them the p-glycoprotein (8-10). Our cocktail is the first one that includes 10 probes designed to assess the activity of the major CYPs, a phase II enzyme, two transporters and a component of renal function. The CYP concerned (CYP 1A2, 2C9, 2C19, 2D6 and 3A4) are the major ones interacting with xenobiotics and especially drugs since they have been reported to interact with 85% (10% for 1A2, 25% for 2C, 15% for 2D6 and 35% for 3A4) of 3486 drugs (17).

The phase II enzymes, *i.e.* uridine diphosphoglucuronosyl transferases UGT 1A1/6/9 also assessed are of major relevance in drug metabolism since UGT catalyzes 35% of conjugation reactions involving human drugs (18). In addition, UGT1A1/6 or /9 are involved in the metabolism of several drugs such as buprenorphine, etoposide, morphine, mycophenolic acid, retigabine (UGT1A1), morphine, valproic acid (UGT1A6) and diclofenac, cotinine, ibuprofen, nicotine, raloxifene (UGT1A9) (19). Acetaminophen is a probe of choice in determining the activities of UGT1A1/6/9 because of its availability, the extensive literature on its role as a UGT probe in humans, and high metabolic activity and glucuronide metabolite production.

Regarding drug transporters, digoxin was previously used in a few cocktails to assess the effect on P-gp activity of an antiretroviral regimen (9), a mixed inhibitor/inducer treatment (8) and HIV infection (10). When digoxin is given orally, the lowest plasma concentrations correspond to the highest P-gp activity (10) since P-gp effluxes the drug from tissues. To assess the OATP1B1 cotransporter activity, we selected rosuvastatin as a probe because it is weakly metabolized (76.8 to 90% excreted unchanged) (20) and its pharmacokinetics are closely related to OATP1B1 genetic polymorphism, as suggested by the potential correlation between the OATP1B1 genetic polymorphisms and altered rosuvastatin pharmacokinetics in Korean populations (21). We had previously discussed the use of rosuvastatin in the CIME cocktail as a probe of the OATP1B1 transporter (12), however it is worth noting that rosuvastatin pharmacokinetics could also depend on BCRP since OATP and BCRP work both in the decrease of plasma exposure (21, 22). As for digoxin, the lowest plasma concentrations

correspond to the highest OATP1B1 activity excreting the drug into the bile. To our knowledge, rosuvastatin has never been used in a cocktail of DMPK probes.

Finally, during the CIME cocktail design, it was decided to assess components of the renal function. Endogenous compounds (*i.e.* creatinine, cystatine C) can be used for this purpose but do not reflect active secretion and/or reabsorption. If necessary, these endogenous biomarkers can be quantified by other means than the cocktail approach. We therefore chose memantine to assess active renal secretion and pH-dependent reabsorption, since its plasma pharmacokinetics correlates with urine pH and it is not metabolized (23). This assessment is in line with guidance on evaluation of the pharmacokinetics of medicinal products in patients with impaired renal function (EMA 2004: *characterization of impairment of glomerular filtration and renal tubular secretion by measurement of renal function*). Moreover, urinary dextrometorphan-based metrics for CYP2D6 activity are influenced by urinary pH (24) and considering the metrics of memantine, it might lead to lower variability. To our knowledge this kind of probe has not been used previously.

Pharmacokinetics of probes and metabolites

In this study, we were able to estimate the main PK parameters for both the substrates and metabolites of the CIME cocktail, except for 3-methoxymorphinan (metabolite of dextromethorphan through CYP 3A4) and 4-hydroxy-midazolam (metabolite of midazolam through CYP 3A4) for which most of our data were under the limit of quantification (0.1 and 1 ng/ml, respectively).

Regarding caffeine and paraxanthine, residual pre-administration concentrations were observed despite the 24-h restriction of caffeine, as already reported in other studies (25, 26). Thus we introduced an individualized correction for combined substrate/metabolite concentration data in order to eliminate the impact of these residual concentrations on the PK parameters and on the phenotypic index. We demonstrated that this MR is not very sensitive to initial perturbation (geometric mean of relative difference with and without correction is less than 2%), in agreement with the experimental work of Perera et al. (26). Our correction therefore will not be applied in subsequent studies, without increasing the 24h restriction of caffeine.

One main issue when developing a new cocktail, especially with as many as 10 different probes, is the possibility that substrates cause drug-drug interactions (6). The CIME cocktail was designed to limit interactions

by: 1) choice of low doses, and 2) selection of well-known substrates with no or few predictable interactions based on the Metabolism and Transport Drug Interaction Database (<http://depts.washington.edu/didbase/>) and a literature search. Caffeine, dextromethorphan, midazolam, omeprazole and tolbutamide are part of published cocktails for which lack of interactions were demonstrated (27-29). It has been reported that omeprazole increases digoxin bioavailability beyond its toxicity threshold (30), but the combination of 1 mg digoxin and 10 mg omeprazole did not lead to clinically significant increase in digoxin C_{max} or in AUC in another study (31). In addition, in a cocktail where probes are given simultaneously and in a single dosing this interaction is unlikely and was not seen in our results: geometric mean digoxin C_{max} was 0.5 ng/ml with a highest C_{max} at 0.8 ng/ml, which remains below the toxic plasmatic threshold (2.5 ng/ml (16)). Finally, although the ten probes of the CIME cocktail were not administrated alone in this study, their PK were extensively described in literature and are in agreement with parameters estimated in our study. A detailed, although non-exhaustive, comparison of published values when drugs are administrated alone with our estimations is briefly discussed molecule by molecule in supplementary information. Taken together, these results indicates that competition in probe absorption (no decrease in C_{max}), perturbations of their metabolism through CYP 2D6, 1A2, 2C19, 2C9, 3A4, 2C8 and UGT-mediated metabolism, or alteration of their transport by P-gp and OATP, and renal tubular reabsorption, are limited if not nonexistent.

Phenotype assessment and genotype/phenotype correlation

The final aim of the CIME cocktail is to phenotype individuals with respect of their metabolism. Although it is not plausible to draw conclusion with only 10 healthy volunteers, we preliminary investigated the variability of the phenotypic indexes and the ability of the CIME cocktail to discriminate between different CYP2D6 genotypes. This trial included a poor metabolizer (Subject 4) for CYP2D6, as assessed by genotyping. The ratio of plasma dextromethorphan AUC_{∞} to dextrorphan AUC_{∞} (DM/DX) was clearly in agreement. The MR values in 8/10 subjects suggest that they are rapid metabolizers, also in agreement with their genotype. Interestingly for these eight subjects, MR values were systematically smaller for the 4 subjects with no mutation (subjects 1, 6, 7, 10; MR = 0.06 – 0.14) compared with subjects with one deficient allele (subjects 2, 3, 5, 8; MR = 0.22-0.39), strongly suggesting that the CIME cocktail is able to detect genotypic differences. The remaining subject

(subject 9) exhibited an extensive metabolizer phenotype based on DM/DX MR (MR = 1.5), in agreement with the genotype. However, for this subject, DM concentrations were high (although much lower than those of the poor metabolizer) as were DX concentrations, with a slow elimination of the latter. An explanation of these high DM levels could be related to a higher DM absorption, whereas DM absorption is usually quite low, *i.e.* around 10% (32). In this subject, a low activity of intestinal P-gp and/or CYP3A could be hypothesized. Thanks to the CIME cocktail, it is possible to examine both P-gp and CYP3A4 activity in this subject with respect to the other nine (despite the small number of volunteers participating in the study). Regarding P-gp, no major differences in the digoxin PK parameters were noted for this subject. For CYP3A, the ratio midazolam/1-hydroxy-midazolam is superior to all other subjects. Thus, these high DM concentrations (despite a normal DM/DX ratio) should be related to a low activity of intestinal CYP3A. Furthermore, these results show the great value of such a cocktail in defining a complete phenotype of an individual, taking into account the links between all of these DMPK enzymes.

Further work

In the present study, we demonstrated that CIME cocktail safety was good, that our selected low doses (ranging from 6 to 50% of the commonly used therapeutic doses) allow sufficient circulating levels for accurate bioanalytical determination, that PK parameters can be determined and that CIME cocktail can detect inter-individual variation, in agreement with genotype and published data. To convert these prospective results into a clinical, routinely-used, phenotypic test, the next developments will consist 1) in verifying the CIME cocktail sensitivity after action of well-known inhibitors or inducers for the addressed enzymes and, importantly, 2) in identifying a few relevant sampling times (typically two or three, common for all the molecules) at which phenotypic indexes are highly correlated to the PK parameters based on a rich design (*e.g.* one sampling time between 4 and 8h for caffeine (6)). To reach these goals, we plan to perform in the next future a larger clinical trial with more volunteers receiving the CIME cocktail before and after dosing with enzymes modulators. The increased number of data will enable us to develop accurate compartmental population models and maximum *a posteriori* bayesian estimators (33) that will be used as relevant limited sampling for future routine use of the CIME test.

4. Methods

Study design

This was a single center, open-label pilot, phase I prospective clinical study conducted at the Bichat-Claude Bernard Hospital Clinical Investigation Center (Paris, France). The study protocol was reviewed and approved by the French Medicine Agency (AFSSAPS) and the Ile-de-France VI Committee for the Protection of Human Subjects participating in Biomedical Research . The legal sponsor was INSERM. The study was carried out in accordance with Declaration Helsinki, followed the International Conference on Harmonization Guidelines for Good Clinical Practice, and was registered at ClinicalTrials.gov (NCT01188525). Written informed consent was obtained from all volunteers before any study procedure and after adequate explanations of the aims, methods, and potential hazards of the study.

After an overnight fast, CIME cocktail molecules (acetaminophen 60 mg, repaglinide 0.25 mg, dextromethorphan 18 mg, digoxin 0.25 mg, memantine 5 mg, midazolam 4 mg, omeprazole 10 mg, rosuvastatin 5 mg, tolbutamide 10 mg, caffeine 73 mg) were orally administered with 200 mL of water at approximately 8:00 AM (Table 4). Subjects remained fasted for 2 h after cocktail administration. They were required to remain at the clinical investigation center within 12 hours following the cocktail administration and to come back to the center at day 1, day 2, day 3 and day 7 post-administration for both safety follow-up and PK sampling purposes. Between each visit, subjects were asked to complete a questionnaire reporting any encountered adverse effects (AE) and consumption of caffeine-containing product or nonstudy medication.

Subjects

Male and female volunteers 18-45 years were eligible if they were in good health, based on medical history, physical examination, and standard laboratory tests including hematology, serum chemistry, urinary analysis and 12-lead electrocardiogram (ECG). Other inclusion criteria included: a body mass index of 19-30 kg/m²; negative urine test for cannabinoids, opioids and amphetamines; no history and no presence of drug or alcohol abuse; negative urinary pregnancy test and the use of a non-oral contraceptive method for women with childbearing potential. Medications (including oral contraceptives) were not permitted within 7 days prior to

the drug administration and during the study, except for a single use of an analgesic for a mild event. Consumption of St John's Wort-, grapefruit- or caffeine-containing products was prohibited within 24h prior to the CIME cocktail administration and during the study.

CYP2D6 and 2C19 genotype assessment

Specific genotypes for CYP with known polymorphisms were not considered as an inclusion or an exclusion criterion. However genotyping of genomic DNA from blood samples was performed for each included subject to assess their metabolizing status of CYP2D6 and CYP2C19.. All volunteers provided written informed consent and approved the sampling and pharmacogenetic analysis. Genotyping was performed in the Hôpital Européen Georges Pompidou, Paris. Genomic DNA was extracted from peripheral blood leukocytes using the QiaAmp DNA mini Kit (Qiagen, Courtaboeuf, France) according to the recommendations of the manufacturer. The SNPs CYP2D6*3 (2549delA, rs35742686),*4 (1846G>A, rs3892097), and *6 (1707delT, rs5030655); CYP2C19*2 (681G>A, rs4244285) and *17 (-806C>T;) were identified using Taq Man® Drug Metabolism Genotyping Assays (Applied Biosystems, Courtaboeuf, France).The CYP2D6 gene deletion (CYP2D6*5) and duplication (CYP2D6*1X or, *2XN) were analyzed by long PCR (the PCR protocol and primers are available on request).The prediction of CYP2D6 and CYP2C19 phenotypes was based on the data from “The Human Cytochrome P450 (CYP) Allele Nomenclature Database” (www.cypalleles.ki.se/).

Safety assessment

During the twelve-hour hospitalization and between each follow-up visit, volunteers were instructed to complete a diary card with description of all symptoms experienced since the last visit. Causality of AEs and CIME cocktail was determined by the principal investigators.

Clinical examination and laboratory tests (hematology and serum chemistry) were performed at screening (baseline) and on day 7 post-administration (end-point). Values outside the clinical range corresponding to healthy individuals and considered as clinically relevant by the principal investigator were reported as an AE.

Vital signs, 12-lead electrocardiogram and physical examination were regularly conducted within the twelve-hour hospitalization and at each follow-up visit (days 1, 2, 3 and 7).Blood glucose level was monitored at hours

4 and 12 post-administration. Real-time monitoring of digoxin (AxSYM process) was also performed on-site on all the 12h blood samples collected following cocktail administration.

PK assessment

Blood samples were collected in heparinized tubes before and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12h following administration, as well as each control visit (24, 48, 72 and 168 h following administration). Blood was centrifuged and plasma was collected in two separate aliquots stored at -80°C until analysis.

Probes and metabolites were assayed by means of an LC-MS/MS method previously developed and validated (12). For last value below the LLQC in the ascending phase of the PK profile and for the first value below the LLQC in the descending phase, a value of one-half of the quantification limit was used; remaining data below the LLQC were excluded.

The PK was estimated by means of non-compartmental analysis (NCA) using an in-house PK module developed in the R statistical software and cross-validated with WINNONLIN® (Pharsight, Mountain View, CA) commercial software. Concentrations of substrates and metabolites and nominal sampling time were used to estimate PK parameters individually in each volunteer. PK measures were maximum observed concentration (C_{max}), area under the plasma concentration-time curve (AUC_{∞}), terminal elimination half-life ($t_{1/2}$), apparent clearance (CL/F) and apparent volume of distribution (V_d/F).

Phenotype assessment

For each target of the CIME cocktail, we determined phenotyping indexes (PI), consisting in substrate AUC_{∞} to metabolite AUC_{∞} ratio (MR) for CYP 1A2 (caffeine to paraxanthine), 2D6 (dextromethorphan to dextrorphan), 2C9 (tolbutamide to 4-hydroxy-tolbutamide), 2C19 (omeprazole to 5-hydroxy-omeprazole), 3A4 (midazolam to 1-hydroxy-midazolam) and UGT (acetaminophen to acetaminophen glucuronide) and in probe AUC_{∞} for CYP 2C8 (repaglinide), OATP (rosuvastatin), P-gp (digoxin) and renal tubular reabsorption (memantine). A review of the literature indicates that these indexes were reflective of the systemic clearance of the probe drug and hence the activity of the CYP pathway under investigation (6). A high PI indicates a low activity of CYP and transporters. A high PI for renal function indicates high tubular reabsorption.

Statistical analyses

The PK parameters, phenotypic indexes and safety data were summarized using descriptive statistics (geometric mean, range). Inter-individual variability was expressed as a coefficient of variation, calculated as follows: $(\text{standard deviation}/\text{mean}) \times 100$.

The non-parametric Wilcoxon matched-pairs signed-ranks test was used to compare safety clinical values at baseline and on day 7 post-administration. A p-value less than 0.05 was considered statistically significant.

Analyses were done using the R statistical software.

Study highlights

What is the state of current knowledge? Cocktails of probes are efficient tools used to assess CYP activities. Some previously developed cocktails have been used either *in vitro* or in volunteers and patients. However, none of these studies included assessment of phase II enzymes, transporters and active renal excretion.

What question did this study address? This study assessed the clinical tolerability and pharmacokinetics of the drugs in the CIME cocktail and their relevant metabolites.

What does this study add and how might it change clinical pharmacology and therapeutics? This study describes the first use in healthy volunteers of a safe and efficient phenotyping cocktail composed of 10 probes assessing six CYP activities, one phase II enzymes, two transporters and active renal excretion. Such a wide spectrum cocktail will enable to phenotype subjects included in clinical trial, to assess more efficiently *in vivo* drug-enzyme interactions and to phenotype patients for treatment individualization.

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Conflict of interest/Disclosure

None

Author Contributions

N. Lenuzza writes the main part of the manuscript, participated in the development of the PK module and was in charge of the data analyses.

G. Nicolas carried out the bioanalysis of the clinical samples.

E. Thévenot participated in the writing of the clinical study protocol and in the development of the PK module for data analysis.

S. Troncale participated in the study design and in the development of the PK module for data analysis.

O. Videau developed and validated the bioanalytical method.

C. Narjoz was responsible for the validation of genetic data and participated in discussions.

M.A. Lorient gave advice on genetic analyses and participated in the final discussions and writing.

L. Becquemont participated in the study design and gave advice on probe selection.

F. Mentré participated in the study design and the PK interpretation.

C. Funck-Brentano participated in the study design and gave advice on probe selection.

L. Alavoine was the clinician which performed the clinical study

P. Arnaud was responsible for the setting up and the fabrication of the tolbutamide capsules and participated in discussions.

M. Delaforge participated in the writing of the clinical study protocol and of the manuscript and is the coordinator of the clinical project.

X. Duval was the clinical study PI. He was responsible for cocktail administration to the volunteer and follow-up. He participated in the study design and participated in the study results interpretation.

H. Bénech participated to the study design, writing of the clinical study protocol and of the manuscript, bioanalysis supervision and raw data validation and publication writing. More generally, he was responsible for the CIME project coordination.

All authors have worked on the draft of the manuscript and have approved the final version.

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Table and Figure legends

Table 1: Effect of the CIME cocktail on healthy volunteers

Data are expressed as geometric mean (CV%)(min-max).

Baseline = inclusion visit or just before the cocktail administration when clinical values were available

End-point=7 days post-administration.

Table 2: Estimated PK parameters for each substrate and their quantifiable metabolite(s).

Tabulated values are geometric means, CV% and range.

Table 3: Estimated phenotypic indexes for each target (CYP, transporters, renal function) of the CIME cocktail. A high metabolic ratio (substrate AUC to metabolite AUC) indicates a low activity of CYP 2C19, 1A2, 2D6, 2C9, 3A4 and UGT. For transporters, a high probe AUC indicates a low activity. High memantine AUC indicates high tubular reabsorption.

Table 4: Description of the CIME cocktail

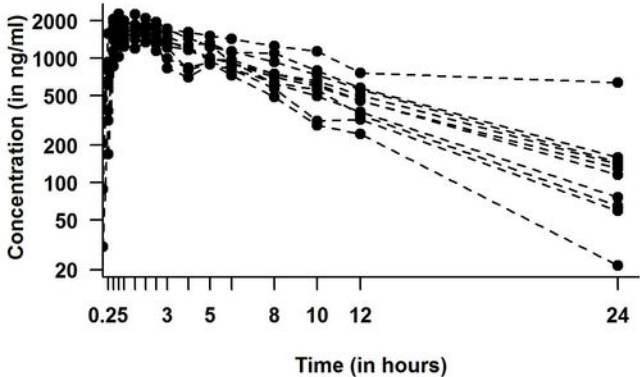
Molecules are listed according to their order of administration.

Figure 1A: Individual plasma concentrations for caffeine and paraxanthine (CYP1A2), dextromethorphan and dextrorphan (CYP2D6), midazolam and 1-hydroxy-midazolam (CYP3A4)

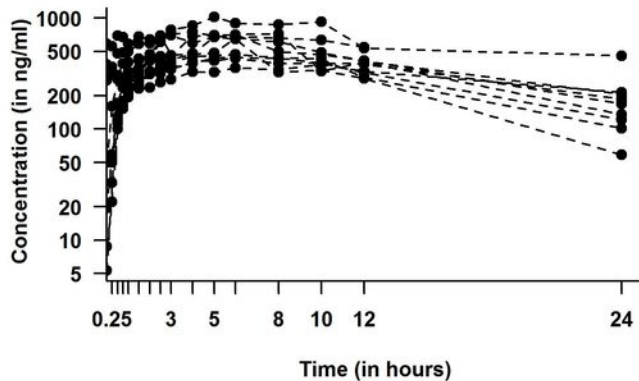
Figure 1B: Individual plasma concentrations for omeprazole, 5-hydroxy-omeprazole (CYP2C19) and omeprazole sulfone, tolbutamide and 4-hydroxy-tolbutamide (CYP2C9) and repaglinide (CYP2C8)

Figure 1C: Individual plasma concentrations for acetaminophen and acetaminophen glucuronide (UGT1A6/1A9), rosuvastatin (OATP1B1) and memantine (tubular renal reabsorption) and digoxin (P-glycoproteine)

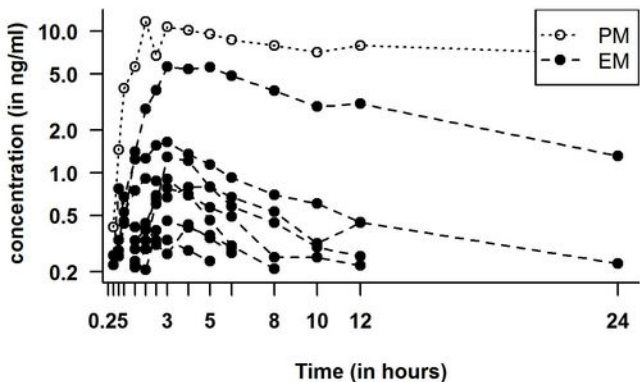
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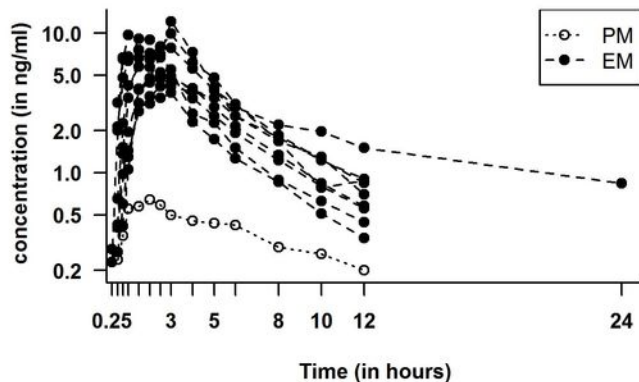
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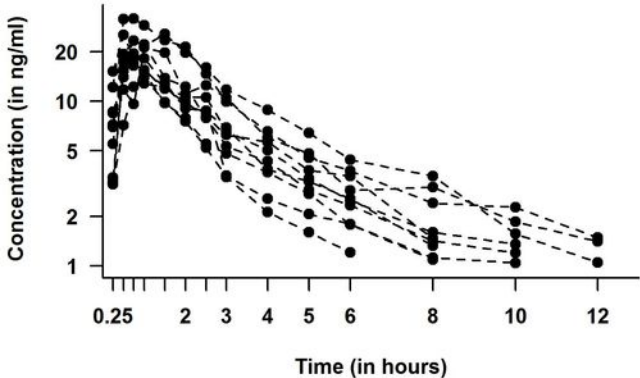
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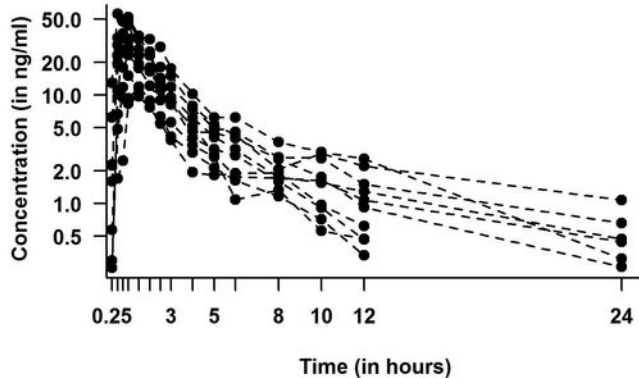
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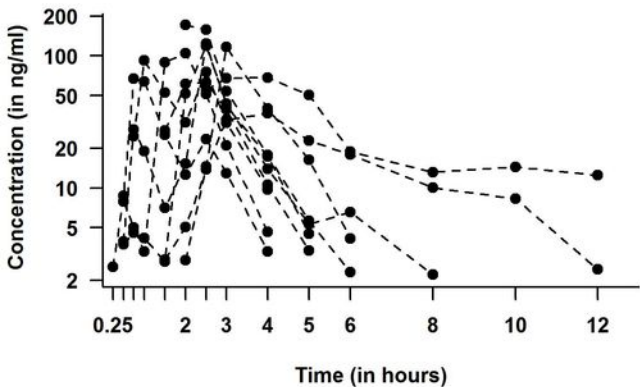
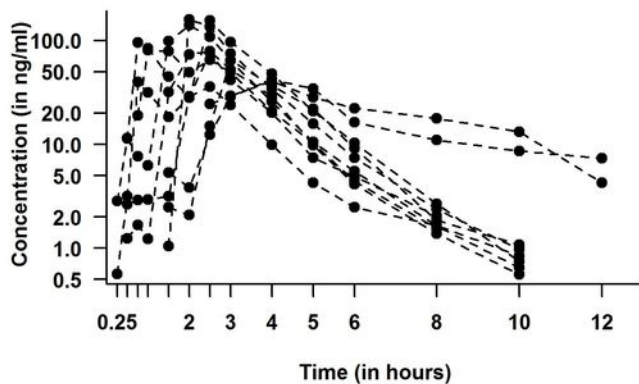
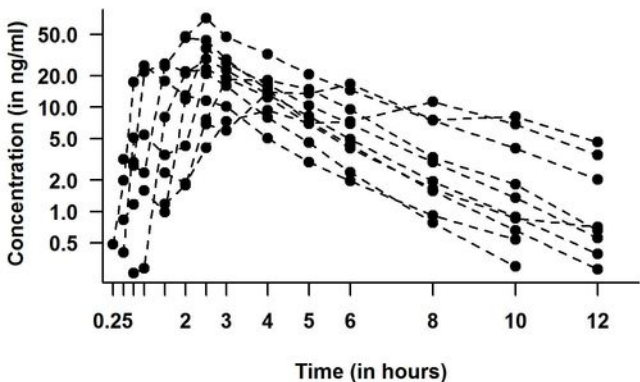
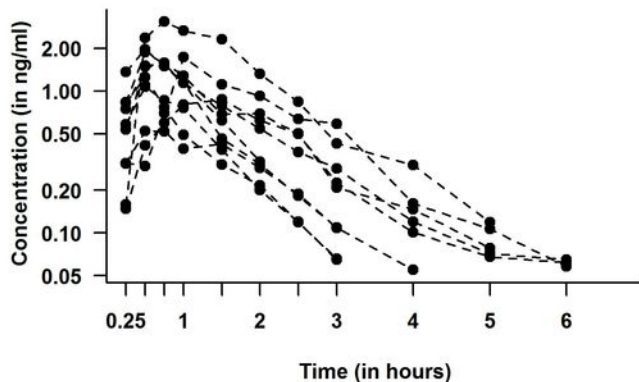
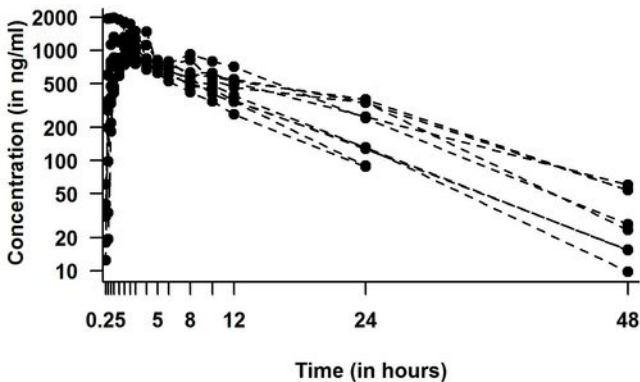
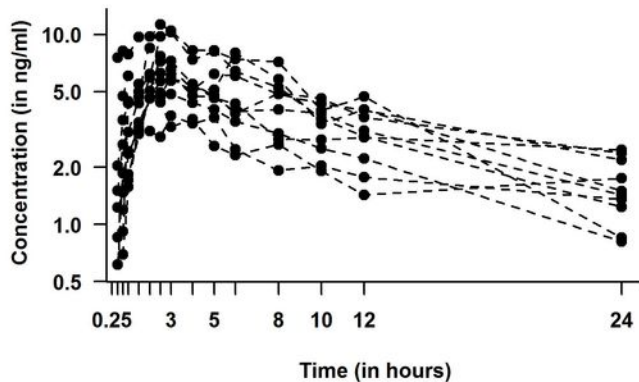


Midazolam

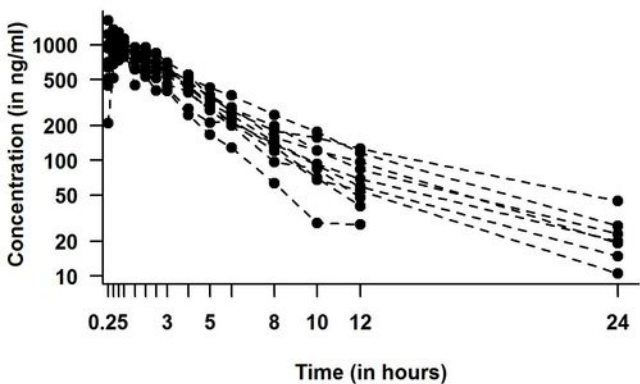


1-hydroxy-midazolam

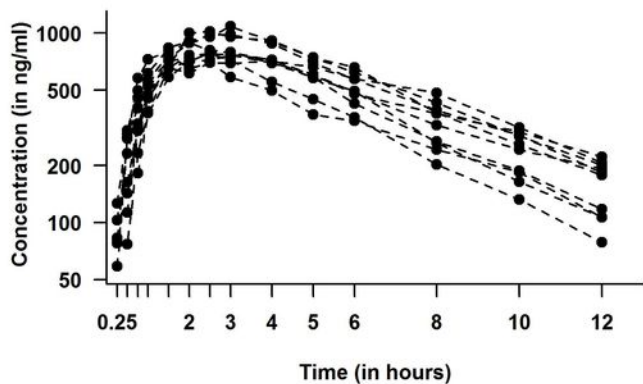


Omeprazole**5-hydroxy-omeprazole****Omeprazole sulfone****Repaglinide****Tolbutamide****4-hydroxy-tolbutamide**

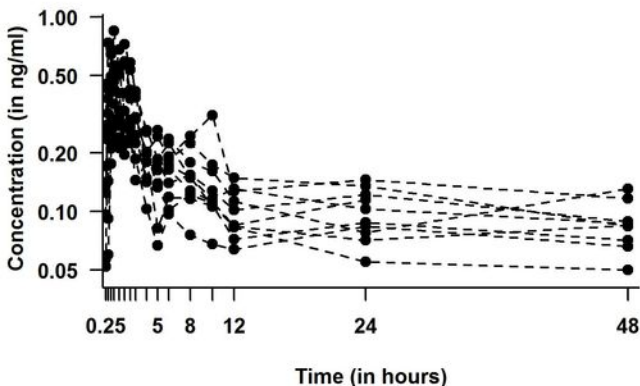
Acetaminophen



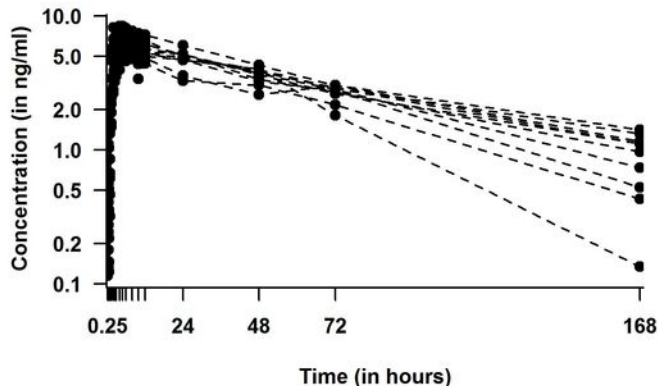
Acetaminophen glucuronide



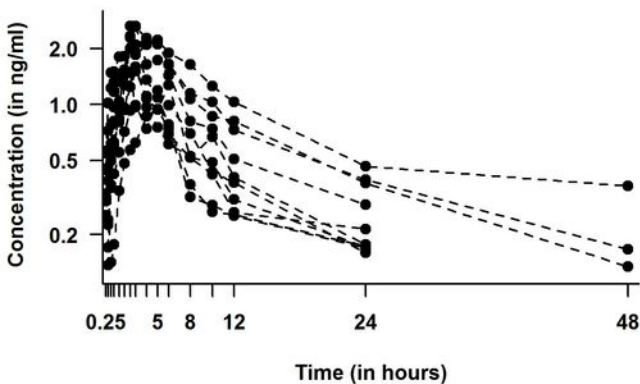
Digoxin



Memantine



Rosuvastatin



Tables & Figures

Table 1: Effect of the CIME cocktail on healthy volunteers

Data are expressed as geometric mean (CV%)(min-max). Baseline = inclusion visit or just before the cocktail administration when clinical values were available. End-point=7 days post-administration.

	Unit	Baseline (n=10) (t=0h, before cocktail administration)	End-point (n=10)
CARDIOVASCULAR SYSTEM			
<i>Sitting systolic blood pressure</i>	mmHg	118 (6.9%) (103- 127)	112 (7.0%) (101-125)
<i>Sitting diastolic blood pressure</i>	mmHg	70 (10.2%) (55- 78)	64 (9.8%) (55-74) *
<i>Heart rate</i>	bpm	62 (15.2%) (45- 84)	67 (7.3%) (60-75)
HEMATOLOGY (inclusion visit)			
<i>Prothrombin rate</i>	%	98 (3.3%) (91-100)	97 (5.5%) (83-100)
<i>Leukocytes</i>	/l ($\times 10^9$)	6.3 (27.3%) (4.2-8.9)	5.9 (20.7%) (4.1-8.0)
<i>Red blood cells</i>	/l ($\times 10^{12}$)	4.79 (9.4%) (4.09-5.50)	4.5 (8.9%) (3.90-5.10) *
<i>Hemoglobin</i>	g/dl	14.4 (8.6%) (12.5-16.7)	13.7 (9.1%) (12.0-15.6) *
<i>Hematocrit</i>	%	41.6 (8.0%) (36.9-47.4)	39.9 (8.3%) (25.3-42.2) *
<i>Mean corpuscular volume</i>	fl	86.3 (3.3%) (82.9-92.0)	86.7 (3.0%) (84.1-92.0)
<i>Mean corpuscular hemoglobin</i>	pg	29.8 (4.2%) (28.1-32.3)	29.8 (4.0%) (28.3-32.2)
<i>Mean corpuscular hemoglobin concentration</i>	g/dl	34.5 (2.5%) (33.2-35.9)	34.4 (1.7%) (33.5-35.3)
<i>Platelets</i>	/l ($\times 10^9$)	221 (17.2%) (171-283)	229 (14.4%) (177-280)
<i>Neutrophils</i>	/l ($\times 10^9$)	3.5 (39.3%) (2.1-5.9)	3.3 (26.5%) (2.1-4.7)
<i>Eosinophils</i>	/l ($\times 10^9$)	0.11 (62.2%) (0.03-0.29)	0.11 (67.3%) (0.03-0.34)
<i>Basophils</i>	/l ($\times 10^9$)	0.03 (42.1%) (0.01-0.06)	0.03 (57.5%) (0.02-0.08)
<i>Lymphocytes</i>	/l ($\times 10^9$)	2.0 (24.5%) (1.6-3.2)	1.8 (25.4%) (1.26-2.96)
<i>Monocytes</i>	/l ($\times 10^9$)	0.50 (28.8%) (0.33-0.79)	0.47 (24.4%) (0.32-0.67)
KIDNEY FUNCTION (BIOCHEMISTRY) (inclusion visit)			
<i>NA+</i>	mmol/l	140 (1.3%) (138-143)	141 (1.3%) (138-144)
<i>K+</i>	mmol/l	3.9 (7.7%) (3.5-4.4)	4.1 (8.2%) (3.4-4.7)
<i>Creatinine</i>	μ mol/l	77 (14.8%) (54-102)	76 (16.8%) (57-95)
<i>CPK</i>	U/l	11 (36.2%) (58-83)	121 (27.6%) (85-174)
LIVER FUNCTION (inclusion visit)			
<i>ALAT</i>	U/l	19 (46.4%) (9-39)	19 (33.9%) (14-36)
<i>ASAT</i>	U/l	24 (13.4%) (21-32)	25(19.6%) (17-35)
<i>Alkaline phosphatases</i>	U/l	60 (24.1%) (40-77)	62 (23.9%) (42-86)
<i>Gamma GT</i>	U/l	15 (33.9%) (9-23)	16(30.5%) (11-24)

* $p < 0.05$, as compared with baseline (Wilcoxon-matched-pairs signed-ranks test)

Tables & Figures

Table 2: Estimated PK parameters for each substrate and their quantifiable metabolite(s).

Tabulated values are geometric means, CV% and range.

		C_{max} (ng/ml)	AUC_{inf} (ng.h/ml)	t_{1/2} (h)	CL/F (l/h)	V_d/F (l)
Acetaminophen	n = 10	1103 (20%) (895-1630)	4682 (24%) (2998-6461)	6.0 (31%) (3.5-9.2)	12.8 (26%) (9.3-20.0)	112 (22%) (74.8-157)
Acetaminophen glucuronide	n = 10	848 (15%) (711-1085)	6661 (22%) (5082-9901)	4.9 (41%) (3.9-11.0)	-	-
Caffeine (before correction)	n = 10	1766 (13%) (1472-2262)	16031 (42%) (10235-35744)	5.9 (44%) (3.5-13.7)	4.6 (30%) (2.0-7.1)	38.7 (14%) (30.3-46.6)
Paraxanthine(before correction)	n = 10	603 (31%) (383-1023)	10418 (46%) (7518-25046)	7.4 (44%) (1.7-16.3)	-	-
Caffeine (after correction)	n = 10	1655 (17%) (1291-2262)	14907 (33%) (9623-27667)	5.9 (44%) (3.5-13.6)	4.9 (29%) (2.6-7.6)	41.7 (18%) (30.3-53.2)
Paraxanthine (after correction)*	n = 9	539 (28%) (376-810)	9790 (36%) (7518-19541)	8.7 (36%) (5.4-17.0)	-	-
Dextromethorphan						
EM	n = 9	0.96 (118%) (0.430-5.62)	7.40 (161%) (2.18-80.7)	4.8 (79%) (1.56-17.1)	2431 (84%) (223-8261)	16835 (48%) (2489-34020)
PM (subject 4)	n = 1	11.73	622	39.7	28.9	1655
Dextrorphan						
EM	n = 9	6.86 (40%) (3.8-12.2)	37.5 (27%) (20.5-54.2)	4.8 (34%) (3.7-9.0)	-	-
PM (subject 4)	n = 1	0.643	7.69	10.4	-	-
Digoxin	n = 10	0.50 (36%) (0.27-0.85)	11.2 (47%) (5.14-23.5)	46.8 (54%) (17.0-107.6)	22.3 (52%) (10.63-48.64)	1506 (25%) (887-2310)
Memantine	n = 10	7.0 (17%) (4.9-8.4)	527 (21%) (364-649)	56.8 (35%) (24.7-99.1)	9.48 (24%) (7.70-13.73)	777 (21%) (487-1121)
Midazolam	n = 10	20.6 (25%) (13.9-31.8)	60.0 (39%) (31.9-110)	2.9 (65%) (1.4-7.6)	66.7 (37%) (36.5-126)	275 (39%) (155-509)
1-hydroxy-midazolam	n = 10	37.1 (52%) (9.67-56.1)	79.1 (51%) (35.7-165)	6.1 (39%) (3.9-10.4)	-	-
Omeprazole	n = 10	88.7 (41%) (36.7-172)	157 (42%) (84.7-336)	0.9 (89%) (0.4-3.4)	63.9 (35%) (29.7-118)	81.0 (66%) (39.1-208)
5-hydroxy-omeprazole	n = 10	84.3 (47%) (40.9-160)	201 (28%) (124-279)	1.6 (93%) (0.9-6.9)	-	-
Omeprazole sulfone	n = 10	26.4 (58%) (11.3-71.0)	89.7 (43%) (47.1-198)	1.9 (39%) (1.3-3.7)	-	-
Repaglinide	n = 10	1.4 (46%) (0.5-3.1)	2.12 (54%) (1.03-5.36)	0.8 (53%) (0.3-2.0)	118 (47%) (46.7-243)	143 (38%) (53.3-257)
Rosuvastatin	n = 10	1.8 (30%) (1.1-2.6)	20.8 (59%) (12.8-56.8)	15.3 (58%) (10.1-44.0)	241 (40%) (88.1-390)	5325 (33%) (3134-8801)
Tolbutamide	n = 10	1287 (27%) (914-1976)	13977 (28%) (8976-21017)	8.2 (18%) (5.8-11.1)	0.72 (29%) (0.48-1.11)	8.4 (21%) (6.2-10.8)
4-hydroxy-tolbutamide	n = 10	6.7 (36%) (3.7-11.3)	100 (24%) (69.2-135)	11.1 (21%) (8.2-14.5)	-	-

Tables & Figures

Table 3: Estimated phenotypic indexes for each target (CYP, transporters, renal function) of the CIME cocktail. A high metabolic ratio indicates a low activity of CYP 2C19, 1A2, 2D6, 2C9, 3A4 and UGT. For transporters, a high PI indicates a low activity. Regarding memantine, high PI memantine indicates high tubular reabsorption.

Target	Substrate (metabolite)	Metrics	Geometric mean (CV%) (min-max)	
CYP2C19	Omeprazole (5-OH-omeprazole)	AUC ratio	0.78 (38%) (0.48-1.33)	n = 10
CYP1A2	Caffeine (Paraxanthine)	AUC ratio (no correction)	1.54 (21%) (0.98-2.11)	n = 10
		AUC ratio (correction)	1.52 (17%) (1.16-1.97)	n = 9
UGT	Acetaminophen (Acetaminophen glucuronide)	AUC ratio	0.70 (29%) (0.42-1.10)	n = 10
CYP2D6	Dextromethorphan (Dextrorphan)	AUC ratio	EM 0.20 (129%) (0.05-1.49)	n = 9
			PM (Subject 4) 80.9	n = 1
CYP2C9	Tolbutamide (4-OH-tolbutamide)	AUC ratio	140 (40%) (80.5-253)	n = 10
CYP3A4	Midazolam (1-OH-midazolam)	AUC ratio	0.76 (83%) (0.34-2.48)	n = 10
CYP2C8	Repaglinide	AUC substrate	2.12 (54%) (1.03-5.36)	n = 10
OATP	Rosuvastatin	AUC substrate	20.8 (59%) (12.8-56.8)	n = 10
P-gp	Digoxin	AUC substrate	11.2 (47%) (5.1-23.5)	n = 10
Tubular absorption	Memantine	AUC substrate	527 (21%) (364-649)	n = 10

Tables & Figures

Table 4: Description of the CIME cocktail

Molecules are listed according to their order of administration.

Molecule	Target	Dosage	Formulation
Acetaminophen	UGT	60 mg	2 ml per os
Repaglinide	CYP2C8	0.25 mg	½ tablet of 0.5 mg
Dextrometorphan	CYP2D6	18 mg	12 ml per os
Digoxin	P-gp	0.25 mg	1 tablet of 0.25 mg
Memantine	Tubular reabsorption	5 mg	0.5 g per os
Midazolam	CYP3A4	4 mg	0.8 ml per os
Omeprazole	CYP2C19	10 mg	1 enteric-coated tablet of 10 mg
Rosuvastatin	OATP	5 mg	1 tablet of 5 mg
Tolbutamide	CYP2C9	10 mg	Magisterial preparation per os
Caffeine	CYP1A2	73 mg	1 bag of instant coffee in warm water