Impact of Acute Inflammation on Cytochromes P450 Activity Assessed by the Geneva Cocktail

Camille Lenoir[1,2], Youssef Daali[1,2], Victoria Rollason[1], François Curtin[1], Yvonne Gloor[1], Marija Bosilkovska[1], Bernhard Walder[3], Cem Gabay[4], Michael John Nissen[4], Jules Alexandre Desmeules[1,2], Didier Hannouche[5] and Caroline Flora Samer[1,*]

Cytochromes P450 (CYP) are subject to important interindividual variability in their activity due to genetic and environmental factors and some diseases. Limited human data support the idea that inflammation downregulates CYP activities. Our study aimed to evaluate the impact of orthopedic surgery (acute inflammation model) on the activity of six human CYP. This prospective observational study was conducted in 30 patients who underwent elective hip surgery at the Geneva University Hospitals in Switzerland. The Geneva phenotyping cocktail containing caffeine, bupropion, flurbiprofen, omeprazole, dextromethorphan, and midazolam as probe drugs respectively assessing CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A activities was administered orally before surgery, day 1 (D1) and 3 (D3) postsurgery and at discharge. Capillary blood samples were collected 2 hours after cocktail intake to assess metabolic ratios (MRs). Serum inflammatory markers (CRP, IL-6, IL-1β, TNF-α, and IFN-γ) were also measured in blood. CYP1A2 MRs decreased by 53% ($P < 0.0001$) between baseline and the nadir at D1. CYP2C19 and CYP3A activities (MRs) decreased by 57% ($P = 0.0002$) and 61% ($P < 0.0001$), respectively, with the nadir at D3. CYP2B6 and CYP2C9 MRs increased by 120% ($P < 0.0001$) and 79% ($P = 0.018$), respectively, and peaked at D1. Surgery did not have a significant impact on CYP2D6 MR. Hip surgery was a good acute inflammation model as CRP, IL-6, and TNF-α peak levels were reached between D1 and day 2 (D2). Acute inflammation modulated CYP activity in an isoform-specific manner, with different magnitudes and kinetics. Acute inflammation may thus have a clinically relevant impact on the pharmacokinetics of these CYP substrates.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ There is a high interindividual variability in cytochromes P450 (CYP) activities due to genetic and environmental factors, as well as some diseases. Limited human data supports the hypothesis that inflammation may downregulate CYP activities.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ What is the impact of acute inflammation triggered by elective hip surgery on the activity of the six major CYP isoforms in humans?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Acute inflammation (hip surgery model), had an impact on CYP activities in an isoform-specific manner, with different magnitudes and kinetics. Our results showed that patients who underwent hip surgery had lower activity of CYP1A2, CYP2C19, and CYP3A. In contrast, CYP2B6 and CYP2C9 activity increased after surgery, whereas variations in CYP2D6 activity were not significant for the duration of the study.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ A greater awareness of the impact of surgery on the pharmacokinetics of drugs metabolized by CYP could help improve drug efficacy and safety in the postoperative setting.

[1]Division of Clinical Pharmacology and Toxicology, Department of Anaesthesiology, Pharmacology, Intensive Care and Emergency Medicine, Geneva University Hospitals, Geneva, Switzerland; [2]Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Geneva, Switzerland; [3]Division of Anaesthesiology, Department of Anaesthesiology, Pharmacology, Intensive Care and Emergency Medicine, Geneva University Hospitals, Geneva, Switzerland; [4]Division of Rheumatology, Department of Medicine, Geneva University Hospitals, Geneva, Switzerland; [5]Division of Orthopaedics and Trauma Surgery, Department of Surgery, Geneva University Hospitals, Geneva, Switzerland. *Correspondence: Caroline Flora Samer (Caroline.Samer@hcuge.ch)

Received July 17, 2020; accepted December 2, 2020. doi:10.1002/cpt.2146

Cytochromes P450 (CYP) are the major drug metabolic enzymes, predominantly expressed in the liver.[1] Among the 57 identified CYP, only a few contribute to drug metabolism with 6 isoforms, namely CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A, metabolizing 90% of marketed drugs.[1] The relative importance of the clearance mechanisms mediated by these isoenzymes range from 46% carried out by members of the CYP3A family, to 16% by CYP2C9, 12% by CYP2C19 and 2D6, 9% by CYP1A, and 2% by CYP2B6.[1] Interindividual variability in CYP activity has been observed as a result of genetic and environmental factors or different disease states.[1]

Genetic polymorphism and/or drug interactions (CYP inhibitors or inducers) can markedly alter drug response, with potential adverse drug reactions (ADRs) and even contribute to the removal of drugs from the market because of unexpected ADR.[1] The ADRs are the fourth leading cause of death in the United States.[2] They trigger hospitalizations or extend hospital stay, whereas being probably preventable in up to three quarters of cases.[2]

Data are further accumulating to point out that the activity of most of the CYPs can either increase or decrease in the presence of endogenous substances, such as proinflammatory cytokines, which can also lead to pharmacokinetic changes and significant drug-drug interactions. Cytokines are intercellular messengers that play a critical role in mediating inflammatory responses and can be additive, synergistic, or inhibitory with each other.[3] Interleukin (IL)-6 is a prototypic proinflammatory cytokine that is directly associated with the degree of inflammation and tissue injury.[4]

Data from *in vitro* and animal models as well as more limited human data support the hypothesis that inflammatory responses are associated with significant reduction in CYP activities.[5] This may alter hepatic clearance of drugs not limited by blood flow.[6] Several mechanisms have been proposed to explain CYP activities' modulation by acute and chronic inflammatory states but the predominant one involves CYP gene expression downregulation by proinflammatory cytokines, such as IL-1, IL-6, and tumor necrosis factor (TNF)-α.[7]

In vitro and animal studies have demonstrated CYP3A downregulation with reduction of mRNA levels.[6,8] In rodents, an acute inflammatory response is associated with a decrease in CYP3A11 mRNA hepatic expression and the causative role of each individual cytokine in CYP3A repression has been studied.[6,8] Moreover, in human hepatocytes cultures, the inducible expression of CYP3A by rifampicin was shown to be suppressed by IL-6.[6,8] In humans, CYP3A activity reduction was maximal 3 days postsurgery with a decrease of 20–60% from baseline levels, depending on the type of surgery.[9] Furthermore, a negative correlation was observed between CYP3A activity and IL-6 peak levels ($r_s = -0.54$, $P = 0.03$).[9] A prospective study in 40 patients with biopsy-proven advanced malignancies showed that the acute-phase response as assessed by C-reactive protein (CRP) levels > 10 mg/L was associated with an average 30% reduction of CYP3A4 activity ($P = 0.0062$).[10] However, the area under the curve (AUC) of atorvastatin, a CYP3A4 substrate, was not modified by cardiac surgery.[11]

The mechanism by which CYP3A gene expression is downregulated by cytokines suggests that the activity of other CYPs could be similarly modulated. Indeed, a key factor appears to be the interplay between inflammatory signaling pathways and transcription factors.[12] Different mediators and transcription factors have been shown to be involved in the regulation of different CYP genes, such as NF-κB, AP-1, SP-1, CAR, PXR, TLR-4, CCAAT enhancer binding proteins family, hepatocyte nuclear factor, and signal transducer and activator of transcription families.[12]

Three case reports describe patients stable on clozapine therapy who developed clozapine toxicity due to increased clozapine plasma concentrations after an infection and/or an inflammatory process, such as surgery, which may be related to cytokine-mediated inhibition of CYP1A2.[5]

Finally, antipyrine (CYP1A2, 2B6, 2C, and CYP3A substrate) and meperidine (CYP3A substrate) plasma half-lives were both significantly decreased during the acute phase of hepatitis compared with recovery period or healthy subjects, although part of the effect might be caused by liver damage among others.[13,14]

The main clinical consequence and concern of these findings is that an inflammatory process can modify exposure to a previously stable drug regimen, thereby possibly resulting in either an increased incidence of ADRs or a lack of efficacy.[6] We therefore sought to evaluate the effects of elective hip surgery as a model of acute inflammation on the activity of the six major CYPs in hospitalized patients using a phenotyping cocktail approach. Total hip surgery was chosen as a model for acute inflammation as it is known to be associated with a significant inflammatory response.[15]

METHODS

Study protocol

This study was a prospective open label observational study investigating the impact of elective hip surgery on the activities of 6 major CYPs, namely CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A. Study protocol (No. 2016-02232) was approved by the regional research ethics committee of the canton of Geneva (CCER) and registered on the US National Institutes of Health clinical trials registry (NCT03262051). Written informed consent was obtained from all patients prior to initiation of any study procedure. This clinical trial was carried out in compliance with the principles of the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice Guidelines.

Study population

Participants were recruited during the pre-operative anesthesia visit for an elective hip surgery scheduled at the Geneva University Hospitals, over a period of 16 months. Eligible patients underwent an elective surgery for hip osteoarthritis and were older than 18 years of age. Exclusion criteria included pregnancy, breastfeeding, and allergy to any of the components of the Geneva cocktail (caffeine, flurbiprofen, omeprazole, bupropion, dextromethorphan, fexofenadine, and midazolam) as well as severe cardiac failure, severe edema or ascites, severe chronic obstructive pulmonary disease or pulmonary embolism requiring oxygen, renal impairment (defined as serum creatinine concentrations > 1.5 × upper limit normal), hepatic impairment (defined as transaminases, bilirubin, gamma glutamyl transferase > 2 × upper limit normal), HIV infection, active cancer, uncontrolled infection, or inflammatory arthritis. Moreover, comedications were systematically screened and patients taking CYP inhibitors or inducers were excluded, using the Lexi-Interact drug interaction checker and the Geneva table of CYP substrates, inhibitors, and inducers.[16,17] Proton pump inhibitor use was allowed in the postoperative setting, as it is a routine prescription after surgery in our hospital that could thus not be excluded. Esomeprazole was the only

proton pump inhibitor administered to the study subjects. The linear mixed model was thus adjusted for esomeprazole intake as it is a well-known CYP2C19 inhibitor.

The primary objective was to measure the variation in the activity of six major CYPs post hip surgery.

Genotyping of CYP2D6, CYP2B6, CYP2C9, and CYP2C19

The method has previously been described in detail in the literature.[18] Briefly, genomic DNA was extracted from EDTA whole blood samples using the QIAamp DNA blood mini kit (Qiagen, Hombrechtikon, Switzerland). Genotyping was performed using TaqMan OpenArray genotyping assays (Life Technologies Corporation, Carlsbad, CA) on a QuantStudio 12K Flex Real-time PCR System (Thermo Fisher Scientific, Rochester, NY). Single-nucleotide polymorphisms used to assess the CYP genotype are listed in **Table S1**. CYP2D6 gene duplication were also assessed with the TaqMan Copy Number Assay Hs00010001 with RNase P as references (Thermo Fisher Scientific). AlleleTyper Software (Thermo Fisher Scientific) was used to translate genetic pattern information from genotyping (Single-nucleotide polymorphisms) and copy number assay to pharmacogenomic gene-level star (*) nomenclature. Translational tables (Thermo Fisher Scientific and PharmGKB) were used to determine genotype for each CYP (star allele nomenclature).

Phenotyping

The metabolic ratio (MR) of 6 CYPs (1A2, 2B6, 2C9, 2C19, 2D6, and 3A) was measured before surgery (D0), day 1 (D1) and day 3 (D3) after surgery and at discharge. Phenotype assessment was performed using the orally administrated probe substrates contained in the Geneva cocktail (caffeine 50 mg, CYP1A2; bupropion 20 mg, CYP2B6; flurbiprofen 10 mg, CYP2C9; omeprazole 10 mg, CYP2C19; dextromethorphan 10 mg, CYP2D6, and midazolam 1 mg, CYP3A). The absence of mutual drug-drug interactions within the Geneva cocktail was previously demonstrated and bupropion is used at such a low dose that no effect on CYP2D6 activity is demonstrated.[19] The cocktail was also previously validated using dried blood spots as a sampling method.[20] Capillary blood samples were collected 2 hours after drug administration in a fasting patient and dried blood spots were stored at –20°C in a sealable plastic bag until analysis, as previously described.[21]

Phenotypic classification was based on MR (defined as the concentration of the metabolite divided by the concentration of the substrate), according to a validated method using liquid chromatography tandem mass spectrometry quantification.[20,22,23] Patients were classified as poor metabolizers (PMs), normal metabolizers (NMs), and ultra-rapid metabolizers (UMs) according to their MRs, as well as intermediate metabolizers for CYP2D6. Threshold values used for phenotype assessment are detailed in **Table S2**.[20,21]

Inflammatory marker levels

Serum levels of IL-6, CRP, TNF-α, IL-1β, and IFN-γ were measured early in the morning, prior to surgery (D0), the first 3 days postsurgery (D1, D2, and D3), and at discharge. The routine concentrations of CRP were measured from lithium heparin whole blood sample, directly after blood collection using latex enhanced immunoturbidimetry. Blood samples underwent centrifugation at 2,000 g and 4°C for 10 minutes and serum samples were stored at –80°C until analysis. Cytokines serum levels were measured using a validated Fluorokine MAP Cytokine Multiplex Elisa assay.

Statistical analysis

A sample size of 30 subjects was required in order to detect a difference of 30% in CYP activity with a power of 80% and an α-value of 5%. All statistical analyses were performed using the IBM SPSS Statistics software version 25 (Chicago, IL) and a *P*-value < 0.05 was considered as statistically significant. Means ± SDs were used to describe continuous variables.

Table 1 Mean MRs ± SD of the six CYP isoforms during the study course (baseline, D1, D2, D3, and discharge; *n* = 30

Isoform	MRs parameters ([Mean] ± SD)	D0	D1	*P* value	D3	*P* value	Discharge	*P* value
CYP1A2	[paraxanthine]/[caffeine]	0.406 ± 0.174	0.190 ± 0.095	< 0.0001	0.207 ± 0.075	< 0.0001	0.264 ± 0.111	0.106
CYP2B6	[OH-bupropion]/[bupropion]	1.591 ± 1.069	3.501 ± 1.613	< 0.0001	3.728 ± 2.309	< 0.0001	4,199 ± 1.988	0.002
CYP2C9	[OH-flurbiprofen]/[flurbiprofen]	0.043 ± 0.021	0.077 ± 0.077	0.018	0.057 ± 0.026	0.002	0.063 ± 0.036	0.007
CYP2C19	[OH-omeprazole]/[omeprazole]	0.760 ± 0.485	0.688 ± 0.745	0.488	0.324 ± 0.509	0.0002	0.564 ± 0.720	0.085
CYP2D6	[dextrorphan]/[dextromethorphan]	1.217 ± 1.459	0.902 ± 0.981	0.334	0.946 ± 1.063	0.330	0.608 ± 0.509	0.062
CYP3A	[OH-midazolam]/[midazolam]	0.888 ± 0.539	0.797 ± 0.359	0.252	0.337 ± 0.125	< 0.0001	0.336 ± 0.095	0.0001

P-values were calculated in comparison with baseline.
D, day; MRs, metabolic ratios.

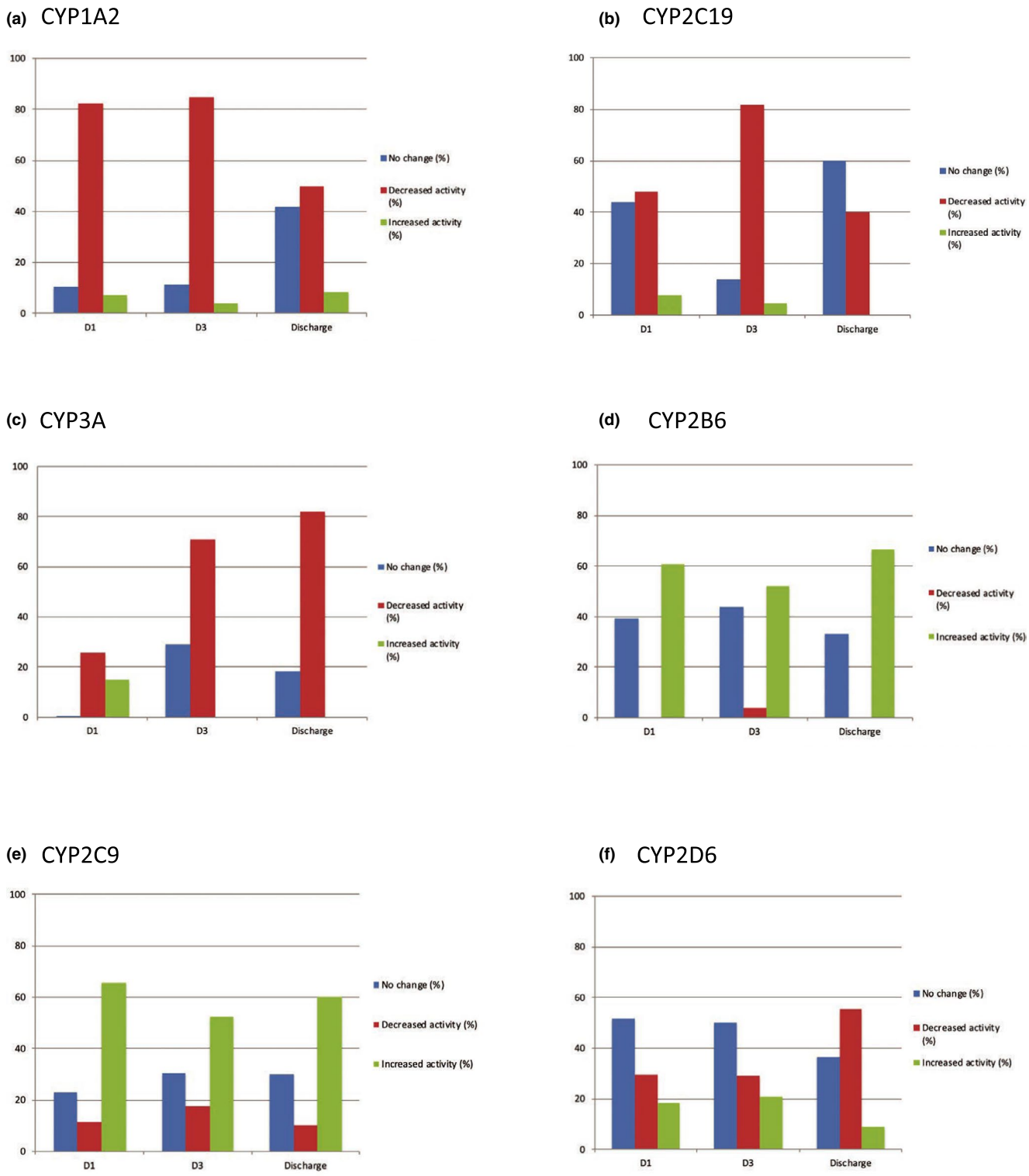

Figure 1 Percentage of patients (n = 30) demonstrating CYP phenoconversion at day (D)1, D3, and discharge: (**a**) CYP1A2, (**b**) CYP2C19, (**c**) CYP3A, (**d**) CYP2B6, (**e**) CYP2C9, and (**f**) CYP2D6

Comparisons of MRs and levels of inflammatory markers before and after surgery were expressed in percentages and analyzed using a paired t-test.

Spearman's rank correlations were used to assess correlation between CYP MRs and inflammatory markers levels, as well as gender, age, body mass index (BMI), or length of surgery. A linear mixed model was built taking into account the repetition of measurements in the same patients as a function of time, to assess the factors (covariables) influencing CYP activities (dependent variables), such as inflammatory markers, BMI, age (continuous variables), as well as surgery, gender, esomeprazole intake, or smoking status (binary variables).

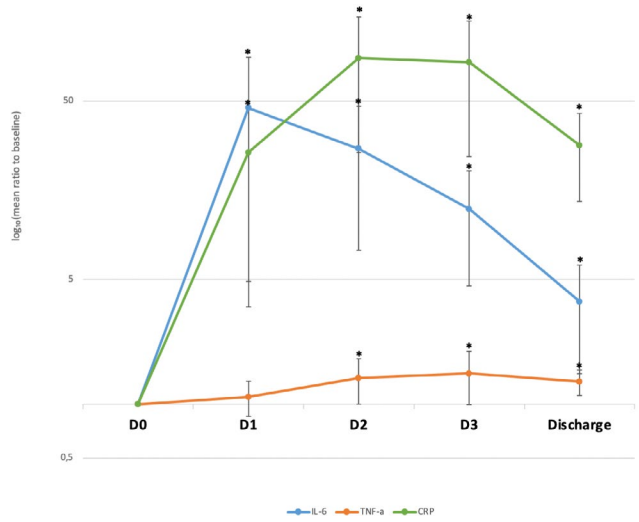

Figure 2 Log_{10} ratio to baseline levels of CRP, IL-6, and TNF-α at baseline, day (D)1, D2, D3, and discharge (n = 30). Error bars represent SD. The P-values were calculated in comparison with baseline, *P < 0.05

RESULTS

Demographic

Thirty White subjects were included with a mean age of 68 ± 11 years and BMI of 27 ± 6. Eighteen subjects (60%) were women. Two patients with type II diabetes were included. The mean duration of surgery was 91 ± 34 minutes, ranging from 54 to 220 minutes. The mean hospital duration after surgery was 4 ± 1 day, ranging from 2 to 6 days. None of the subjects had any drug safety concerns.

CYP activity before and after surgery

The activities of the 6 major CYPs before and after surgery are reported in **Table 1**. CYP1A2 MRs decreased by 53.2% (P < 0.0001), with a maximal effect at D1 postsurgery. CYP2C19 and CYP3A activities decreased by 57.5% (P = 0.0002) and 61.3% (P < 0.0001), respectively, between baseline and the nadir at D3 postsurgery. Conversely, CYP2B6 and CYP2C9 MRs increased by 120.1% (P < 0.0001) and 79.1% (P = 0.018), respectively, and were maximal at D1. The decrease of CYP2D6 MRs (50.0%) did not reach statistical significance before discharge (P = 0.062). None of the MRs of the six CYPs returned to normal levels prior to discharge.

Phenoconversion

All patients were genotyped and allelic frequencies for each CYP studied are presented in **Table S3** with predicted phenotypes.

The phenoconversion of CYP1A2, CYP2C19, CYP2D6, and CYP3A was assessed in phenotypic non-PM subjects after surgery. The phenotypic switch after surgery from NM to PM or from UM to NM was seen in 82% of subjects for CYP1A2 and CYP2C19 and 70% for CYP3A4 (**Figure 1a–c**). Concerning CYP2B6 and CYP2C9, as the MRs increased after surgery, UM subjects were excluded from the analysis. Sixty percent and 65% of patients had a phenotypic switch from either PM to NM or NM to UM, respectively (**Figure 1d,e**). Regarding CYP2D6, 55% of patients had a phenotypic switch at discharge (NM to intermediate metabolizer; **Figure 1f**).

Proinflammatory markers

The effects of surgery on inflammatory markers (CRP, IL-6, and TNF-α) exposure are shown in **Figure 2**. IL-6 serum levels peaked at D1, whereas TNF-α and CRP peaked at D2 postsurgery. IL-1β and IFN-γ were undetectable.

Circulating levels of TNF-α correlated with CRP (r = 0.542, P = 0.001) and IL-6 (r = 0.435, P = 0.013) levels. As expected, the correlation between circulating levels of IL-6 and CRP was even stronger (r = 0.613, P = 0.0001).

No correlation was demonstrated with gender, age, or BMI (P > 0.05 for all). Serum levels of IL-6 correlated with duration of hip surgery (r = 0.433, P = 0.017).

Variables that influenced change in CYP activity

No statistically significant correlation was demonstrated between extreme CYP MRs and peak levels of inflammatory markers.

Table 2 shows the correlation between MRs of each CYP isoforms and corresponding IL-6, TNF-α, and CRP serum levels.

A linear mixed model was built to assess the factors correlated with CYP activities, such as inflammatory markers, BMI, gender, age, esomeprazole intake, or smoking status (**Table 3**).

Several variables were significantly correlated with the activity of some CYPs, such as surgery (CYP1A2, 2B6, 2C9, and 3A), CRP (CYP2C19 and CYP3A), IL-6 (CYP3A), BMI (CYP1A2 and 2C19), and esomeprazole intake (CYP2C19). Age, gender, ethnicity, and smoking status were not correlated with CYP variations.

DISCUSSION

We assessed the impact of acute inflammation (elective hip surgery) on the activity of six major CYPs and demonstrated that surgery modulated CYP activity in an isoform-specific manner, with different magnitudes and kinetics. To our knowledge, this is the first time that CYP activities, other than CYP3A, have been studied in the postoperative setting.

In our study, CYP3A activity decreased by 60% after surgery (maximal after 3 days) and was inversely correlated with surgery and CRP, and positively correlated with IL-6. Previous publications have demonstrated that infection and more broadly inflammation decreased CYP3A activity, and in proportion to the severity of the disease.[5,11,14,24,25] Moreover, authors have shown that CYP3A4 activity was inversely correlated to CRP levels.[26,27] Surgery and cancer have also been associated with decreased CYP3A4 activity and increased serum levels of CRP and IL-6, respectively.[9,10] Moreover, in patients with rheumatoid arthritis, when inflammation was reversed by tocilizumab, an anti-IL-6 receptor antibody, exposure to simvastatin was significantly reduced by half at 1 and 5 weeks after infusion.[5] This is in line with our findings regarding CRP but not IL-6. Comparison of the correlation between CYP activities and IL-6 both before and after inflammation was not assessed in most published studies. A direct correlation over a short period of time would not be necessarily expected, because a time lag between IL-6 levels elevation

Table 2 Correlation (Spearman) among the MRs of the six CYP isoforms and IL-6, TNF-α, and CRP serum levels measured at specific timepoints in the 30 subjects

	CYP1A2	CYP2C19	CYP3A	CYP2B6	CYP2C9	CYP2D6
IL-6	−0.517 *P = 0.0001*	−0.165 *P = 0.102*	0.022 *P = 0.828*	0.336 *P = 0.001*	0.347 *P = 0.001*	−0.127 *P = 0.209*
CRP	−0.400 *P = 0.0001*	−0.417 *P = 0.0001*	−0.527 *P = 0.0001*	0.447 *P = 0.0001*	0.172 *P = 0.088*	−0.136 *P = 0.180*
TNF-α	0.135 *P = 0.183*	−0.104 *P = 0.308*	−0.296 *P = 0.003*	0.002 *P = 0.985*	−0.009 *P = 0.927*	−0.257 *P = 0.010*

and CYP downregulation could be expected. A 3-day lag after surgery between IL-6 elevation and CYP3A downregulation has already been described.[9] Furthermore, the mean IL-6 peak levels in our study were 1.6-fold to 5.1-fold lower than those previously reported in other types of surgery (peripheral vascular surgery with graft and abdominal aortic aneurysm, respectively).[9] Further investigations would be needed to confirm our results after cardiovascular surgery. If confirmed, other preclinical experiments would be required to understand the pathophysiology behind the association between CRP levels and CYP3A activity using *in vitro* and animal models.

Similarly to our results, many studies found decreased CYP1A2 activity in inflammatory conditions, such as infection or induced-infection models.[5,28] Even though tobacco smoking is a known inducer of CYP1A2, we did not find that smokers' status modulated CYP1A2 activity in our study, probably because of the small number of smokers ($n = 6$) and as smoking is forbidden in the hospital setting.[5] Significant inverse associations have previously been established between IL-6 levels and CYP1A2 activity ($r = -0.5$, $P = 0.0235$) but not with TNF-α, in 16 patients with congestive heart failure.[29] Several case reports have described increased clozapine toxicity or plasma concentration after infection and/or inflammatory processes.[5] The decrease of CYP1A2 activity described in our study confirms that it could be of clinical relevance as a phenoconversion was seen in 82% of patients. These changes in CYP1A2 activity led to increased risk of ADR and required dose adaptation.[30] Some authors reported an association between circulating concentrations of CRP and clozapine.[30,31] These published studies are in agreement with our results, because we found an inverse Spearman's correlation with IL-6 and CRP but not with

Table 3 Standardized variables in the linear mixed model and correlation with the metabolic activity of the six CYP isoforms in the 30 subjects

	CYP1A2	CYP2C19	CYP3A	CYP2B6	CYP2C9	CYP2D6
Surgery	−1.1867 (SE = 0.2215) *P* = 0.0001	0.4685 (SE = 0.2941) *P* = 0.115	−0.5622 (SE = 0.2079) *P* = 0.008	1.1910 (SE = 0.2117) *P* = 0.0001	0.6516 (SE = 0.2699) *P* = 0.018	−0.2428 (SE = 0.1842) *P* = 0.192
IL-6	−0.0935 (SE = 0.0863) *P* = 0.282	0.1004 (SE = 0.0914) *P* = 0.275	0.2902 (SE = 0.0809) *P* = 0.001	−0.1041 (SE = 0.0816) *P* = 0.206	0.0611 (SE = 0.1053) *P* = 0.563	−0.0349 (SE = 0.0700) *P* = 0.619
CRP	−0.0990 (SE = 0.0999) *P* = 0.324	−0.3045 (SE = 0.1062) *P* = 0.005	−0.2757 (SE = 0.0965) *P* = 0.005	−0.0295 (SE = 0.0970) *P* = 0.762	−0.1519 (SE = 0.1220) *P* = 0.216	0.0748 (SE = 0.0879) *P* = 0.398
TNF-α	0.1278 (SE = 0.0977) *P* = 0.198	0.1779 (SE = 0.1136) *P* = 0.123	−0.0333 (SE = 0.1113) *P* = 0.766	−0.0903 (SE = 0.1144) *P* = 0.432	−0.0727 (SE = 0.1206) *P* = 0.549	−0.1826 (SE = 0.1133) *P* = 0.111
BMI	0.2157 (SE = 0.1049) *P* = 0.049	−0.4965 (SE = 0.1261) *P* = 0.0001	−0.1768 (SE = 0.1345) *P* = 0.201	−0.0960 (SE = 0.1514) *P* = 0.531	0.2444 (SE = 0.0011) *P* = 0.056	0.0279 (SE = 0.1997) *P* = 0.890
Age	0.06678 (SE = 0.0962) *P* = 0.493	−0.2008 (SE = 0.1205) *P* = 0.106	0.0393 (SE = 0.1281) *P* = 0.761	−0.0754 (SE = 0.1432) *P* = 0.602	−0.0475 (SE = 0.1192) *P* = 0.693	−0.0432 (SE = 0.1869) *P* = 0.819
Gender (male)	0.0787 (SE = 0.1854) *P* = 0.674	0.0867 (SE = 0.2319) *P* = 0.712	−0.3386 (SE = 0.2530) *P* = 0.194	−0.1041 (SE = 0.2883) *P* = 0.721	0.1157 (SE = 0.2300) *P* = 0.618	−0.2868 (SE = 0.3817) *P* = 0.460
No intake of esomeprazole	n.a.	0.7763 (SE = 0.2737) *P* = 0.006	n.a.	n.a.	n.a.	n.a.
Nonsmoker	−0.1089 (SE = 0.2278) *P* = 0.636	n.a.	n.a.	n.a.	n.a.	n.a.

BMI, body mass index; MRs, metabolic ratios; n.a., not applicable.

TNF-α. However, conflicting results were reported in patients with diabetes.[32,33] In our study, only surgery was inversely correlated with CYP1A2 activity in the linear mixed model, but not cytokines' levels. This means that surgery triggered changes, other than an increase in cytokines' levels that could be responsible for the downregulation of CYP1A2 activity. It is indeed well-known that CYP1A2 is easily modulated by endogenous compounds and xenobiotics. BMI was also positively correlated to CYP1A2 activity in our study, but at the limit of significance. This has never been shown before in the literature.

We demonstrated that CRP was inversely correlated to CYP2C19 MR but that surgery, IL-6, and TNF-α were not. Other possible changes caused by surgery are therefore not involved in the downregulation of CYP2C19 activity. In patients with type 2 diabetes, CYP2C19 activity significantly decreased by half ($P = 0.001$) as compared with controls and multivariate models showed that IFN-γ and TNF-α partly explained these variations.[32] Moreover, CRP and IL-6 were significantly and inversely associated with CYP2C19 activity.[29,34] Other authors showed that CYP2C19 predicted and measured phenotype in patients with cancer were statistically discordant, but no significant correlations between the levels of any individual cytokine (CRP, IL-1β, IL-1α, IL-6, TNF-α, and TGF-β) were found.[5]

In our study, BMI was associated with a significant CYP2C19 activity reduction, which is supported by the literature.[35,36] In fact, the rate of high on-treatment platelet reactivity to clopidogrel was significantly associated with higher BMI as well as CYP2C19 loss-of-function alleles (LoFAs) carrier (*2 or *3).[35] In LoFA noncarriers with overweight/obesity, clopidogrel-aspirin therapy was not efficient in reducing the risk of stroke recurrence as compared with LoFA noncarriers with low/normal weight.[36] Again, we expect CYP2C19 activity decrease to be clinically relevant due to the observed phenoconversion in 82% of patients.

In the literature, it is described that cytokines downregulate CYP activity and this is consistent with our results, because we have shown that it is not the increase in cytokines' levels that is responsible of induction of CYP2B6 and 2C9 activities, but other mechanisms induced by surgery. Indeed, surgery was positively correlated to CYP2B6 and 2C9 MRs in our study and not to IL-6, CRP, and TNF-α levels.

We showed that CYP2B6 activity increased from the first day after surgery and that cytokine levels were not correlated to CYP2B6 MR when the model was adjusted to surgery status. Published data rather reported CYP2B6 activity decrease in inflammatory conditions.[32,37] A multivariate model conducted in patients with type II diabetes showed that IFN-γ and TNF-α partly explained these variations and the administration of IFN-α before cyclophosphamide (CP) caused a 63% decrease in its clearance ($P = 0.004$) compared with 24 hours after CP.[32,37] However, CP is a prodrug bioactivated by both CYP3A4 and 2B6.[37] The contribution of decreased CYP3A activity could thus not be ruled out. Hepatic CYP2B genes represent the most inducible CYP isoforms by phenobarbital-type compounds in most mammalian species.[38]

Phenoconversion was observed in 60% of our cohort of patients. One of the major factors that contribute to CYP2B6 modulation, like other inducible CYP, is the regulation of its transcription by several nuclear hormone receptors, such as PXR, CAR, glucocorticoid receptor (GR), and vitamin D receptor, in a direct and/or indirect manner.[38] In addition, CYP2B6 expression is inducible under stress conditions, such as fasting or energy restriction.[38] As cortisol, the glucocorticoid "stress hormone" binds the GRs, and increases under stress conditions, such as surgery, induction of CYP2B6 by surgery itself via the GR cannot be excluded.[39] In a randomized controlled study conducted in patients with elective hip surgery, cortisol levels indeed changed over time ($P < 0.001$).[40] The GR could also be implicated in CYP2C9 induction.[41]

We established that CYP2C9 activity increased after surgery, and was correlated with IL-6 but not with CRP and TNF-α. Several studies confirmed that the activity of CYP2C9 increased under inflammatory conditions as a consequence of a disease state or exogenous administration of cytokines.[5,32] However, conflicting results have been published, in particular with warfarin and losartan, where increased plasma concentration or bleeding events were reported during inflammation.[5,42,43] Nevertheless, warfarin and losartan are mainly metabolized by CYP2C9, but are also minor substrates of CYP3A4, 2C19, 1A2, and CYP3A, respectively, whose activities were reduced in our study. Moreover, the increase of CYP2C9 activity found in our study could be considered as clinically relevant as phenoconversion was seen in 65% of patients.

We described that CYP2D6 activity did not change significantly in the first 3 days after surgery, but a trend for a 50% decrease was noted at discharge, and inversely correlated with surgery and TNF-α levels. Other authors have also suggested that acute inflammation does not impact on CYP2D6 activity, as well as diabetes (type I, type II, and gestational).[32,33,44–46] In a study conducted in patients with congestive heart failure, TNF-α and IL-6 levels were furthermore not associated with CYP2D6 activity.[29] However, another study showed that CYP2D6 activity (mean urinary dextromethorphan ratio for 4 consecutive days) was significantly higher in HIV-infected patients than in healthy volunteers.[5] Thus, a decrease of CYP2D6 activity could occur at a later stage than that of other isoenzymes and this would be in line with our results where CYP2D6 activity decreased by 50% at discharge. Phenoconversion of CYP2D6 was observed in 55% of our cohort. The clinical relevance of this finding remains to be demonstrated due to the wide variability of CYP2D6 activity.

Three patients were CYP2D6 genotypic PMs in our study, and they were kept in our analysis because the correlation with CYP2D6 MRs were overall not significantly different whether they were included or not in the analysis. Besides, the genotypic activity of their other CYP was normal.

We carefully reviewed the anesthetics and analgesics administrated during the peri-operative period in order to exclude an impact on the activity of CYP, on top of the comedications systematically screened before surgery (exclusion of CYP inhibitors or inducers). None of the anesthetics and analgesics used were known to modulate CYP activity, except for propofol that has been shown to be weak CYP3A inhibitor, mainly

in *in vitro* studies. A double-blind randomized study conducted in 24 patients showed that the impact on midazolam metabolite formation was only statistically significant during the first 30 minutes of anesthesia induction with propofol but not during the 6 hours thereafter,[47] due to the short half-life of the molecule. It is therefore reasonable to exclude a significant impact of these medications administrated in the peri-operative setting on the activity of assessed CYP.

We thus showed that surgery had an impact on CYP in an isoform-specific manner that may have a clinically relevant impact on regular treatment and analgesia after surgery, such as CYP3A, CYP2C9, CYP2C19, and CYP1A2 substrates. In our study, more than 50% of patients were receiving CYP substrate to treat comorbidities and among analgesic drugs, almost three quarters were CYP substrates. Furthermore, these variations in MRs were of different magnitudes and kinetics and were correlated with different inflammatory markers.

Events, such as surgery, trauma, infection, burns, or advanced cancer, have been associated with significant variations in plasma concentration of acute phase proteins.[48] IL-6 cytokine is the key stimulator of acute phase protein production as well as other cytokines, such as IL-1β, TNF-α, and IFN-γ. Moreover, TNF-α and IL-6 promote the transcriptional induction of the CRP gene.[49] This supports our finding of a correlation among IL-6, TNF-α, and CRP. The modest effect of TNF-α found in our study might thus be an indirect effect of IL-6.

Different factors have been shown to influence systemic cytokine levels and some cytokines have extremely brief half-lives, making their detection difficult. In fact IL-1β and IFN-γ are rarely detectable in human serum, except in the case of severe inflammation or after intensive sampling in the perioperative period.[50,51] Authors have shown that IL-6 peak levels were reached 4–48 hours after surgery and fell rapidly after 48–72 hours.[51] CRP levels appear to rise more slowly postoperatively compared with cytokine levels.[52] In a study conducted in the same conditions as ours, CRP level reached its peak 2 days after surgery and remained significantly elevated on the third day after surgery.[15] These findings are in line with our study data.

Similarly to our results, other authors have found a correlation between increased IL-6 levels and the duration of surgery but not with gender after elective hip surgery.[53] Cytokine levels have been shown to increase with age, but we only observed a trend for IL-6 and TNF-α.[54] In accordance with our results, no correlation was found in the literature between cytokine levels and either BMI or gender.[55]

Our study has some limitations. The sample size was relatively small and confirmation of our linear mixed model findings in an additional and/or larger sample is warranted. Moreover, only two patients with type II diabetes were included in our cohort and it was thus impossible to draw any conclusion on the impact of type II diabetes on CYP activities. Furthermore, due to the methodology and statistical analyses used, a correlation between surgery and modulation of CYP activity was shown, but further investigations are needed to strengthen criteria of causation.

To conclude, our results indicate that surgery and acute inflammation have a major impact on the activity of six major CYPs in an isoform-specific manner of different magnitude and velocity. Our findings could thus have a relevant impact on the pharmacokinetics of drugs metabolized by these key drug-metabolizing enzymes and could help improve drug efficacy and safety in the postoperative setting.

SUPPORTING INFORMATION

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

ACKNOWLEDGMENTS

The authors thank Fabienne Doffey-Lazeyras, the Immunology and Clinical Allergology laboratory, the nurses of the division of Orthopaedics and Trauma Surgery, and the presurgery hospitalization unit, and the anaesthesiologists of Geneva University Hospitals for their help and support.

FUNDING

This research was supported by a Geneva University Hospitals (HUG) grant number PRD 5-2018-I.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHORS CONTRIBUTIONS

C.L., V.R., and C.F.S. wrote the manuscript. Y.D., Y.G., M.B., B.W., D.H., C.F.S., C.G., M.J.N., and J.A.D designed the research. C.L. performed the research. C.L., F.C., and C.F.S. analyzed the data. C.L., Y.D., and Y.G. contributed new reagents/analytical tools.

1. Wienkers, L.C. & Heath, T.G. Predicting in vivo drug interactions from in vitro drug discovery data. *Nat. Rev. Drug Discov.* **4**, 825–833 (2005).
2. Giardina, C. *et al*. Adverse drug reactions in hospitalized patients: results of the FORWARD (Facilitation of Reporting in Hospital Ward) Study. *Front. Pharmacol.* **9**, 350 (2018).
3. Rankin, J.A. Biological mediators of acute inflammation. *AACN Clin. Issues* **15**, 3–17 (2004).
4. Reikeras, O., Borgen, P., Reseland, J.E. & Lyngstadaas, S.P. Changes in serum cytokines in response to musculoskeletal surgical trauma. *BMC Res. Notes* **7**, 128 (2014).
5. Shah, R.R. & Smith, R.L. Inflammation-induced phenoconversion of polymorphic drug metabolizing enzymes: hypothesis with implications for personalized medicine. *Drug Metab. Dispos. Biol. Fate Chem.* **43**, 400–410 (2015).
6. Morgan, E.T. Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics. *Clin. Pharmacol. Ther.* **85**, 434–438 (2009).
7. Aitken, A.E., Richardson, T.A. & Morgan, E.T. Regulation of drug-metabolizing enzymes and transporters in inflammation. *Annu. Rev. Pharmacol. Toxicol.* **46**, 123–149 (2006).
8. Morgan, E.T. Regulation of cytochromes P450 during inflammation and infection. *Drug Metab. Rev.* **29**, 1129–1188 (1997).
9. Haas, C.E., Kaufman, D.C., Jones, C.E., Burstein, A.H. & Reiss, W. Cytochrome P450 3A4 activity after surgical stress. *Crit. Care Med.* **31**, 1338–1346 (2003).
10. Rivory, L.P., Slaviero, K.A. & Clarke, S.J. Hepatic cytochrome P450 3A drug metabolism is reduced in cancer patients who have an acute-phase response. *Br. J. Cancer* **87**, 277–280 (2002).
11. Kruger, P.S. *et al*. A preliminary study of atorvastatin plasma concentrations in critically ill patients with sepsis. *Intensive Care Med.* **35**, 717–721 (2009).

12. Ruminy, P. *et al*. Gene transcription in hepatocytes during the acute phase of a systemic inflammation: from transcription factors to target genes. *Inflamm. Res. Off. J. Eur. Histamine Res. Soc. Al* **50**, 383–390 (2001).
13. Burnett, D.A., Barak, A.J., Tuma, D.J. & Sorrell, M.F. Altered elimination of antipyrine in patients with acute viral hepatitis. *Gut* **17**, 341–344 (1976).
14. McHorse, T.S., Wilkinson, G.R., Johnson, R.F. & Schenker, S. Effect of acute viral hepatitis in man on the disposition and elimination of meperidine. *Gastroenterology* **68**, 775–780 (1975).
15. Bjornsson, G.L. *et al*. Inflammatory cytokines in relation to adrenal response following total hip replacement. *Scand. J. Immunol.* **65**, 99–105 (2007).
16. Samer, C.F., Lorenzini, K.I., Rollason, V., Daali, Y. & Desmeules, J.A. Applications of CYP450 testing in the clinical setting. *Mol. Diagn. Ther.* **17**, 165–184 (2013).
17. Interactions médicamenteuses, cytochromes P450 et P-glycoprotéine (P gp) <https://www.hug.ch/sites/interhug/files/structures/pharmacologie_et_toxicologie_cliniques/a5_cytochromes_6_2.pdf>.
18. Broccanello, C., Gerace, L. & Stevanato, P. QuantStudio™ 12K flex OpenArray® system as a tool for high-throughput genotyping and gene expression analysis. *Methods Mol. Biol. Clifton NJ* **2065**, 199–208 (2020).
19. Bosilkovska, M. *et al*. Evaluation of mutual drug-drug interaction within Geneva cocktail for cytochrome P450 phenotyping using innovative dried blood sampling method. *Basic Clin. Pharmacol. Toxicol.* **119**, 284–290 (2016).
20. Bosilkovska, M. *et al*. Geneva cocktail for cytochrome p450 and P-glycoprotein activity assessment using dried blood spots. *Clin. Pharmacol. Ther.* **96**, 349–359 (2014).
21. Lloret-Linares, C. *et al*. Screening for genotypic and phenotypic variations in CYP450 activity in patients with therapeutic problems in a psychiatric setting, a retrospective study. *Pharmacol. Res.* **118**, 104–110 (2017).
22. Bosilkovska, M. *et al*. Simultaneous LC-MS/MS quantification of P-glycoprotein and cytochrome P450 probe substrates and their metabolites in DBS and plasma. *Bioanalysis* **6**, 151–164 (2014).
23. Jerdi, M.C., Daali, Y., Oestreicher, M.K., Cherkaoui, S. & Dayer, P. A simplified analytical method for a phenotyping cocktail of major CYP450 biotransformation routes. *J. Pharm. Biomed. Anal.* **35**, 1203–1212 (2004).
24. Morcos, P.N. *et al*. Influence of chronic hepatitis C infection on cytochrome P450 3A4 activity using midazolam as an in vivo probe substrate. *Eur. J. Clin. Pharmacol.* **69**, 1777–1784 (2013).
25. Latorre, A. *et al*. Clinical management of renal transplant patients with hepatitis C virus infection treated with cyclosporine or tacrolimus. *Transplant. Proc.* **34**, 63–64 (2002).
26. Molanaei, H. *et al*. Inflammation down-regulates CYP3A4-catalysed drug metabolism in hemodialysis patients. *BMC Pharmacol. Toxicol.* **19**, 33 (2018).
27. Molanaei, H. *et al*. Metabolism of alprazolam (a marker of CYP3A4) in hemodialysis patients with persistent inflammation. *Eur. J. Clin. Pharmacol.* **68**, 571–577 (2012).
28. Yamaguchi, A. *et al*. Higher incidence of elevated body temperature or increased C-reactive protein level in asthmatic children showing transient reduction of theophylline metabolism. *J. Clin. Pharmacol.* **40**, 284–289 (2000).
29. Frye, R.F., Schneider, V.M., Frye, C.S. & Feldman, A.M. Plasma levels of TNF-alpha and IL-6 are inversely related to cytochrome P450-dependent drug metabolism in patients with congestive heart failure. *J. Card. Fail.* **8**, 315–319 (2002).
30. Espnes, K.A., Heimdal, K.O. & Spigset, O. A puzzling case of increased serum clozapine levels in a patient with inflammation and infection. *Ther. Drug Monit.* **34**, 489–492 (2012).
31. Darling, P. & Huthwaite, M.A. Infection-associated clozapine toxicity. *Clin. Schizophr. Relat. Psychoses* **5**, 159–160 (2011).
32. Gravel, S., Chiasson, J.-L., Turgeon, J., Grangeon, A. & Michaud, V. Modulation of CYP450 activities in patients with type 2 diabetes. *Clin. Pharmacol. Ther.* **106**, 1280–1289 (2019).
33. Matzke, G.R., Frye, R.F., Early, J.J., Straka, R.J. & Carson, S.W. Evaluation of the influence of diabetes mellitus on antipyrine metabolism and CYP1A2 and CYP2D6 activity. *Pharmacotherapy* **20**, 182–190 (2000).
34. Veringa, A. *et al*. Voriconazole metabolism is influenced by severe inflammation: a prospective study. *J. Antimicrob. Chemother.* **72**, 261–267 (2017).
35. Ma, Q., Chen, G.-Z., Zhang, Y.-H., Zhang, L. & Huang, L.-A. Clinical outcomes and predictive model of platelet reactivity to clopidogrel after acute ischemic vascular events. *Chin. Med. J. (Engl.)* **132**, 1053–1062 (2019).
36. Mo, J. *et al*. Efficacy of clopidogrel-aspirin therapy for stroke does not exist in CYP2C19 loss-of-function allele noncarriers with overweight/obesity. *Stroke* **51**, 224–231 (2020).
37. Hassan, M., Nilsson, C., Olsson, H., Lundin, J. & Osterborg, A. The influence of interferon-alpha on the pharmacokinetics of cyclophosphamide and its 4-hydroxy metabolite in patients with multiple myeloma. *Eur. J. Haematol.* **63**, 163–170 (1999).
38. Wang, H. & Tompkins, L.M. CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. *Curr. Drug Metab.* **9**, 598–610 (2008).
39. Jacques, A., Battle, A.R. & Johnson, L.R.Glucocorticoid receptor (GR). In *Encyclopedia of Signaling Molecules* (ed. Choi, S.), 2121–2126 (Springer International Publishing, Cham, 2018). https://doi.org/10.1007/978-3-319-67199-4_101536.
40. Kwon, Y.S. *et al*. Effects of surgery start time on postoperative cortisol, inflammatory cytokines, and postoperative hospital day in hip surgery: randomized controlled trial. *Medicine* **98**, e15820 (2019).
41. Pascussi, J.M., Gerbal-Chaloin, S., Drocourt, L., Maurel, P. & Vilarem, M.J. The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim. Biophys. Acta* **1619**, 243–253 (2003).
42. Goktaş, M.T. *et al*. Lower CYP2C9 activity in Turkish patients with Behçet's disease compared to healthy subjects: a down-regulation due to inflammation? *Eur. J. Clin. Pharmacol.* **71**, 1223–1228 (2015).
43. Blumenkopf, B. & Lockhart, W.S. Herpes zoster infection and use of oral anticoagulants. A potentially dangerous association. *JAMA* **250**, 936–937 (1983).
44. Hefner, G., Shams, M.E.E., Unterecker, S., Falter, T. & Hiemke, C. Retrospective pilot study for analysis of antidepressant serum concentrations of citalopram and venlafaxine during inflammation. *Pharmacopsychiatry* **48**, 215–218 (2015).
45. Hefner, G., Falter, T., Bruns, K. & Hiemke, C. Elevated risperidone serum concentrations during acute inflammation, two cases. *Int. J. Psychiatry Med.* **50**, 335–344 (2015).
46. Jetter, A. *et al*. Do activities of cytochrome P450 (CYP)3A, CYP2D6 and P-glycoprotein differ between healthy volunteers and HIV-infected patients? *Antivir. Ther.* **15**, 975–983 (2010).
47. Hamaoka, N. *et al*. Propofol decreases the clearance of midazolam by inhibiting CYP3A4: an in vivo and in vitro study. *Clin. Pharmacol. Ther.* **66**, 110–117 (1999).
48. Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340**, 448–454 (1999).
49. Sproston, N.R. & Ashworth, J.J. Role of C-reactive protein at sites of inflammation and infection. *Front. Immunol.* **9**, 754 (2018).
50. Pape, H.C. *et al*. Biochemical changes after trauma and skeletal surgery of the lower extremity: quantification of the operative burden. *Crit. Care Med.* **28**, 3441–3448 (2000).
51. Baigrie, R.J., Lamont, P.M., Kwiatkowski, D., Dallman, M.J. & Morris, P.J. Systemic cytokine response after major surgery. *Br. J. Surg.* **79**, 757–760 (1992).
52. Bergin, P.F. *et al*. Comparison of minimally invasive direct anterior versus posterior total hip arthroplasty based on inflammation and muscle damage markers. *J. Bone Joint Surg. Am.* **93**, 1392–1398 (2011).
53. Minetto, M.A. *et al*. Serum interleukin-6 response to elective total hip replacement surgery. *Int. Orthop.* **30**, 172–176 (2006).
54. Krabbe, K.S., Pedersen, M. & Bruunsgaard, H. Inflammatory mediators in the elderly. *Exp. Gerontol.* **39**, 687–699 (2004).
55. Reikerås, O. & Borgen, P. Activation of markers of inflammation, coagulation and fibrinolysis in musculoskeletal trauma. *PLoS One* **9**, e107881 (2014).