Clinical Confirmation that the Selective JAK1 Inhibitor Filgotinib (GLPG0634) has a Low Liability for Drug-drug Interactions

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Abstract: Objective: The selective Janus kinase 1 inhibitor filgotinib (GLPG0634), which is currently in clinical development for the treatment of rheumatoid arthritis (RA) and Crohn’s disease, demonstrated encouraging safety and efficacy profiles in RA patients after 4 weeks of daily dosing. As RA patients might be treated with multiple medications simultaneously, possible drug-drug interactions of filgotinib with cytochrome P450 enzymes and with key drug transporters were evaluated in vitro and in clinical studies.

Methods: The enzymes involved in filgotinib’s metabolism and the potential interactions of the parent and its active major metabolite with drug-metabolizing enzymes and drug transporters, were identified using recombinant enzymes, human microsomes, and cell systems. Furthermore, filgotinib’s interaction potential with CYP3A4 was examined in an open-label study in healthy volunteers, which evaluated the impact of filgotinib co-administration on the CYP3A4-sensitive substrate midazolam. The potential interaction with the common RA drug methotrexate was investigated in a clinical study in RA patients.

Results: In vitro, filgotinib and its active metabolite at clinically relevant concentrations did not interact with cytochrome P450 enzymes and uridine 5′-diphospho-glucuronosyltransferases, and did not inhibit key drug transporters. In the clinic, a lack of relevant pharmacokinetic drug interactions by filgotinib and its active metabolite with substrates of CYP3A4, as well as with organic anion transporters involved in methotrexate elimination were found.

Conclusion: the collective in vivo and in vitro data on drug-metabolizing enzymes and on key drug transporters, support co-administration of filgotinib with commonly used RA drugs to patients without the need for dose adjustments.

Keywords: Carboxylesterases, CYP450, drug-drug interaction, drug transporters, filgotinib, selective JAK1.

INTRODUCTION

Recent observations suggest inhibition of JAK1 to be largely responsible for the efficacy of JAK inhibitors in patients with immune-inflammatory disease [1]. Filgotinib (GLPG0634) is a JAK1-selective inhibitor with a half maximal inhibitory concentration (IC₅₀) value of 629 nM (267 ng/mL); in human whole blood the compound is 30 times more selective for JAK1 over JAK2 [2]. In preclinical studies, an active filgotinib metabolite with a similar JAK1 selectivity profile but less potent (IC₅₀ = 11.9 µM or 4,529 ng/mL) than the parent compound was identified [3]. In human, exposure to this active metabolite is approximately 16 to 20-fold higher than to the parent filgotinib, which might compensate for the lower potency of the metabolite [4]. Filgotinib is extensively and rapidly absorbed after oral dosing. The rate (Cₘₐₓ) and the extent (AUC) of absorption of filgotinib increased dose proportionally over the anticipated therapeutic dose range (50 to 200-mg daily dose). Consistent with the 6-h elimination half-life of filgotinib, there was no accumulation at steady state. After filgotinib dosing, the concentrations of the main metabolite reached a maximum within 3–5 h and then slowly decreased with an apparent elimination half-life of about 23 h. Filgotinib and its metabolites are predominantly eliminated in urine (>80%). In plasma, urine and feces, the predominant metabolites are the active metabolite and its N-glucuronide derivate.

RA is a chronic inflammatory and degenerative joint disease affecting about 1% of the worldwide adult population, with a higher prevalence in women. Although RA can occur at any age, it usually begins between the ages of 40 and 60. In particular elderly patients are at high risk for adverse events from drug–drug interactions (DDIs) due to chronic disease, physiologic changes associated with aging, and the tendency to use multiple medications [5]. The average older person uses two to six prescription medications and one to three non-prescription medications on a routine basis. The most common mechanism underlying DDI relates to the interplay with cytochrome P450 enzymes (CYP450s), with the inhibition of these enzymes being most often responsible for life-threatening interactions. In addition to these metabolic enzymes, the role drug transporters play in DDI, safety, and effectiveness of drugs has been greatly appreciated in recent years [6]. Transporter-inhibiting drugs can alter the transporter functional activity and/or protein expression, hence causing transporter-specific interactions.
Consequently, the aims of the current studies were to better understand the potential for interaction of filgotinib and its main metabolite with CYP450s as well as with other metabolizing enzymes such as uridine 5’-diphospho-glucuronosyltransferase (UGTs), and with key drug transporters as defined in FDA [7] and EMA [8] guidelines, to provide guidance to the concomitant use of filgotinib and other drugs in patients with chronic inflammatory diseases like RA. The current paper presents the characterization of the key enzymes involved in the metabolism of filgotinib, the in vitro studies investigating interactions of filgotinib and its main metabolite with metabolizing enzymes and drug transporters, and the clinical confirmation of the conclusions from these in vitro studies. Clinical evaluations were done in healthy volunteers for the potential effects of filgotinib on midazolam, a sensitive CYP3A4 substrate, and in RA patients for the interaction with methotrexate, a substrate of organic anion transporters (OAT) and a common treatment in RA. Given the low binding to plasma proteins for both filgotinib and its main metabolite (<40%), interaction potential with metabolizing enzymes and drug transporters was assessed using the total maximal concentration (Cmax) observed at the highest anticipated therapeutic dose (200 mg daily dose) i.e. 1.4 µg/mL (3.4 µM) for filgotinib and 3.8 µg/mL (11 µM) for its main metabolite.

MATERIALS AND METHODS

Materials and Reagents

Human liver microsomes (pooled from 26 or 30 donors) were prepared and characterized at Biopredic International (France) as well as HepaRG™ cells. Human recombinant carboxylesterases (CES1-b, CES1-c, and CES2 transcript variants) and human recombinant UGTs were supplied by BD Bioscience (France). The membrane vesicles overexpressing human ABC transporter (BSEP) were prepared and supplied by SOLVO Biotechnology (Hungary). The CHO cells expressing uptake/SLC transporters were supplied by the University of Zurich (Switzerland) for OATP1B1/3 and by SOLVO Biotechnology (Hungary) for OCT1/2, the transfected MDCKII- BCRP cell monolayers by the University of Greifswald (Germany) and the transfected MDCKII- MDR1 (P-gp) cell monolayers by Creative Cell (Hungary). Human embryonic kidney cell line (HEK-MSRII) transduced with BacMam baculovirus OAT1, OAT3 or OAT4 were prepared and provided by GSK (United Kingdom). All chemicals and reagents used were commercially available and of guaranteed purity.

Identification of the Enzymes Involved in Filgotinib Metabolism

Given the structure of filgotinib and its main metabolites (Fig. 1) it was hypothesized that enzymes other than CYP450s might be involved in filgotinib’s metabolism and in particular hydrolases such as amidases or carboxylesterases. To investigate this hypothesis, filgotinib (30 µg/mL, i.e., 70 µM) was incubated with pooled human liver microsomes for one hour at 37°C with or without nicotinamide adenine dinucleotide phosphate (NADPH). The effect of a non-specific amidase inhibitor, b-(p-nitrophenyl) phosphate (bis-pNPP) [9] on the formation of the metabolite was also assessed. In a second step, the enzymes involved in filgotinib metabolism were further characterized at two drug concentrations (2.6 and 26 µg/mL, i.e., 6 and 60 µM) using human recombinant enzymes (hCES1b, hCES1c and hCES2; 0.1 mg/mL). In both experiments, the concentrations of metabolite formed were monitored by liquid-chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Experiment was conducted in triplicate and the results were expressed as percentage of total amount of metabolite formed.

Experimental in vitro Systems to Assess CYP450 Inhibition

The ability of filgotinib and its major metabolite to inhibit CYP1A2 (enzyme activity: phenacetin O-deethylase; inhibitor: furafylline, 10 µM), CYP2A6 (coumarin 7-hydroxylase; methoxsalen, 1 µM), CYP2B6 (bupropion hydroxylase; thiotepa, 4 µM), CYP2C8 (paclitaxel 6α-hydroxylase; quercetin, 30 µM), CYP2C9 (tolbutamide

Fig. (1). Structure of filgotinib and its major active metabolite.
methylhydroxylase; sulfaphenazole, 5 µM), CYP2C19 (S-mephenytoin 4'-hydroxylase; tranylcypromine, 40 µM), CYP2D6 (dextromethorphan O-demethylation; quinidine, 2 µM), CYP2E1 (chlorozoxazone 6-hydroxylase; disulfiram, 200 µM) and CYP3A4/5 (midazolam 1'-hydroxylase, nifedipine oxidase and testosterone 6β-hydroxylase. ketoconazole, 0.5 µM) was investigated in vitro using pool of human liver microsomes (HLMs). Filgotinib (up to 30 µg/mL, i.e., 70 µM), its metabolite (up to 80 µg/mL, i.e., 224 µM) and reference CYP inhibitors were first incubated for 20 minutes with microsomes (0.3-1.0 mg of protein/mL) in presence of NADPH to investigate potential mechanism-based inhibition. Then, specific probe substrates were added and the incubation was prolonged for 5-30 minutes. Reactions were terminated with acetonitrile or acetonitrile and centrifuged to remove proteins. The experiment was conducted in triplicate.

The ability of filgotinib and its major metabolite to inhibit CYP1A2 (enzyme activity: phenacetin O-deethylase; phenacetin, 200 µM) and CYP2B6 (bupropion hydroxase; bupropion, 100 µM) was investigated in vitro using HepaRG cells, a human hepatoma cell line developed from a differentiated hepatocarcinoma. These cells exhibit morphological similarity to human hepatocytes and also express major CYP-related activities as well as transporters and other liver-specific functions [10, 11]. Cells were incubated with filgotinib (up to 3 µg/mL, i.e., 7 µM) or its metabolite (up to 80 µg/mL, i.e., 224 µM) for 48 hours at 37°C. A reference inducer for each CYP tested was also included at a single concentration: omeprazole (CYP1A2 inducer; 50 µM), phenobarbital (CYP2B6 inducer; 1mM) and rifampicin (CYP3A4 inducer; 10 µM). After incubation, cells were washed and incubated for 2 hours with CYP-specific substrates. The enzyme activities were determined by LC-MS/MS analysis of CYP-dependent activities obtained without test compounds or reference inhibitors. The stability of filgotinib and its main metabolite in HLM was assessed in parallel to the inhibition experiment. No degradation of the two compounds was observed after incubation in the same conditions as for CYP inhibition evaluation. Consequently the inhibition observed could be attributed with certainty to filgotinib or its main metabolite. The experiment was conducted in triplicate.

**Experimental in vitro Systems to Assess CYP 450 Induction**

The potential for filgotinib and its major metabolite to induce CYP1A2 (enzyme activity: phenacetin O-deethylase; CYP-specific substrate: phenacetin, 200 µM), CYP2B6 (bupropion hydroxase; bupropion, 100 µM) and CYP3A4 (midazolam 1'-hydroxylase, midazolam, 50 µM) was investigated in vitro using HepaRG cells, a human hepatoma cell line developed from a differentiated hepatocarcinoma. These cells exhibit morphological similarity to human hepatocytes and also express major CYP-related activities as well as transporters and other liver-specific functions [10, 11]. Cells were incubated with filgotinib (up to 3 µg/mL, i.e., 7 µM) or its metabolite (up to 80 µg/mL, i.e., 224 µM) for 48 hours at 37°C. A reference inducer for each CYP tested was also included at a single concentration: omeprazole (CYP1A2 inducer; 50 µM), phenobarbital (CYP2B6 inducer; 1mM) and rifampicin (CYP3A4 inducer; 10 µM). After incubation, cells were washed and incubated for 2 hours with CYP-specific substrates. The enzyme activities were determined by LC-MS/MS analysis of CYP-dependent metabolite formed and expressed as relative activity corrected by the cell protein contents. The experiment was conducted in triplicate.

**Experimental in vitro Systems to Assess UGT Inhibition**

The ability of filgotinib and its major metabolite to inhibit UGTs present mainly in the liver, i.e., UGT1A1 (inhibitor: ketoconazole, 100 µM; substrate: β-estradiol, 20 µM), UGT1A4 (hecogenine, 10 µM; trifluoperazine, 1 µM), UGT1A6 (troglitazone, 100 µM; deferiprone, 1mM), UGT1A9 (niflumic acid, 5 µM; propofol, 75 µM), and UGT2B7 (fluconazole 50 mM; zidovudine, 70 µM), was investigated in vitro using human recombinant enzymes. Filgotinib (up to 26 µg/mL, i.e., 60 µM), its metabolite (up to 29 µg/mL, i.e., 80 µM) and reference UGT inhibitors were first incubated for 15 minutes with human UGTs (hUGTs) (0.1-0.2 mg/mL) in presence of UDPGA cofactor to active hUGTs before adding probe substrate. Then, specific probe substrates were added and the incubation prolonged for 20-60 minutes. Reactions were terminated with acetonitrile and centrifuged to remove proteins. The experiment was conducted in triplicate. The enzyme activities were determined by the LC-MS/MS analysis of UGT dependent metabolite formed and expressed as percent of the control activities obtained without test compounds or reference inhibitors.

**Experimental in vitro Systems to Assess Drug Transporters Inhibition**

The potential of filgotinib and its major metabolite to inhibit key drug transporters was investigated using assays for cell-based transport (P-glycoprotein [P-gp], breast cancer resistance protein [BCRP]), membrane vesicle transport inhibition (bile salt export pump [BSEP]) or cell-based uptake inhibition for organic anion transporters (OAT1, OAT3, and OAT4), organic cation transporters (OCT1 and OCT2) and organic anion-transporting polypeptide (OATP1B1 and OATP1B3). Uptake experiments were performed on Chinese Hamster Ovary cells overexpressing the transporters of interest except for OATs for which Human Embryonic Kidney MSRII (HEK-MSRII) or Human Embryonic Kidney (HEK293) cells were used. Inhibition was first investigated at 60 µM for both filgotinib and its metabolite for all drug transporters except for OATs (100 µM for filgotinib and 300 µM for its metabolite). In case a significant inhibition was observed, the determination of the IC₅₀ was assessed in a second step. Reference substrates and inhibitors for each drug transporter were used as positive controls. Substrates were quantified by liquid scintillation counting (LSC) or by measuring fluorescence. The experiment was conducted in triplicate.

**Drug-drug Interaction with Midazolam in Healthy Volunteers**

This study was a Phase 1, open-label, drug-drug interaction study to evaluate the effect of multiple oral doses of filgotinib on the single-dose PK profile of midazolam administered in fasted healthy male volunteers (aged 40-60 years, body mass index [BMI]: 18-30 kg/m²). Each volunteer received a single oral dose of midazolam (2 mg as syrup) on 2 occasions (days 1 and 8) and once daily dose of filgotinib (200 mg as powder blend in capsules) from day 2 to day 8. On day 8, the single 2 mg dose of midazolam was administered at the same time as the 200 mg filgotinib dose. Filgotinib was administered once daily for 7 days to ensure that steady state concentrations for filgotinib and its major active metabolite have been reached and that potential enzyme induction could be evaluated. An oral midazolam dose of 2 mg was judged to have minimal pharmacological effects and it is also the recommended oral dose to
investigate the maximal inhibitory effect, considering that the extent of interaction may depend on the dose of the CYP substrate [12]. This study was conducted in accordance with the accepted standards for the protection of subject safety and welfare, and with the principles of the Declaration of Helsinki and its amendments, and was in compliance with Good Clinical Practice. The protocols and informed consents were reviewed and approved by the Institutional Review Board of the investigational center participating in the study.

The sample size based on confidence interval was calculated according to the tables of Diletti et al. [13]. For a risk of 5%, an expected ratio of AUC between 0.95 and 1.05, and a standard error of midazolam AUC of 0.18, a sampling of 17 subjects provide 80% power that the confidence interval (CI) for the ratio of the mean will be contained within the equivalence range of 80% to 125%. In order to have 17 evaluable subjects available, 20 subjects were included in the study. The value of 0.18 of the standard error used in this calculation comes from a previous midazolam interaction study [14].

Blood samples for midazolam pharmacokinetics were collected at predose and 0.25, 0.5, 1, 2, 3, 4, 5, 8, 10, 12 and 24 hours post dose on day 1 (midazolam) and day 8 (midazolam + filgotinib). Plasma was separated in a refrigerated centrifuge (4 - 8°C; 10 minutes at approximately 1,500 g) within 30 minutes after blood collection, and stored at -20°C until analysis. Plasma concentrations of midazolam were determined after liquid-liquid extraction using a validated LC-MS/MS assay. Calibration standard responses were within 30 minutes after blood collection, and stored at -20°C until analysis. Plasma concentrations of midazolam were determined after liquid-liquid extraction using a validated LC-MS/MS assay. Calibration standard responses were linear over the 0.100 to 100 ng/mL using a weighted (1/concentration²) linear regression. The lower limit of quantification was 0.100 ng/mL.

The plasma concentrations of midazolam were analyzed following a non-compartmental approach (WinNonLin®, version 5.3; Pharsight corporation, Mountain View, CA, USA). The peak plasma concentration (Cmax) and time to Cmax (tmax) were directly observed from the data. The terminal elimination rate constant (λz) was reported only when more than three data-points were used in the linear regression determining λz with an adjusted R² value ≥ 0.900. The area under the curve (AUC) was calculated over 24 hours (AUC0-24h) and extrapolated to infinity (AUC0-∞).

An analysis of variance (ANOVA) with subject as a random effect and day as a fixed effect was performed on the following natural logarithm (ln)-transformed parameters of midazolam: Cmax, AUC0-24h, AUC0-∞, and t1/2,λz. Point estimates were calculated for each parameter as the geometric mean ratio of Day 8 (midazolam + filgotinib) relative to Day 1 (midazolam) with corresponding 90% CI. The lack of an interaction on midazolam pharmacokinetics would be demonstrated if the 90%CI for the ratio of adjusted geometric means for Cmax and AUCs fell wholly within the 80-125% interval. SAS® version 9.1 (SAS institute Inc. Cary, NC, USA) at a 0.05 level of significance was used for statistical inferential analyses.

Drug-drug Interaction with Methotrexate in RA Patients

The impact of filgotinib on the pharmacokinetics of methotrexate (MTX) was evaluated during an exploratory, randomized, double-blind, placebo-controlled Phase 2A dose ranging study in patients with active RA and an inadequate response to MTX; patients were administered once daily filgotinib (30, 75, 150, and 300 mg) or placebo for 4 weeks in addition to their stable oral dose of MTX [15]. Sample size calculation for the assessment of the interaction was not carried out since it was not the primary objective of this Phase 2A study. The participation to the MTX pharmacokinetics was left to patient’s willingness. A total of 17 patients spread across the different filgotinib dose groups took part in the drug-drug interaction assessment. This study was conducted in accordance with the accepted standards for the protection of subject safety and welfare, and with the principles of the Declaration of Helsinki and its amendments, and was in compliance with Good Clinical Practice. The protocol and informed consent were approved by local Ethical Committees from the 4 countries involved in the study (Moldova, Ukraine, Russia, and Hungary). All RA patients gave written informed consent prior to study initiation.

Blood samples to assess individual steady state pharmacokinetics of MTX (predose and 1, 2, 3, and 8 hours post MTX dose) were collected before concomitant dosing with filgotinib (day-1) and after 2 or 4 weeks of once daily dosing with filgotinib. Plasma was separated in a refrigerated centrifuge (4 - 8°C; 10 minutes at approximately 1,500 g) within 30 minutes after blood collection, and stored at -20°C until analysis. Plasma concentrations of MTX were determined after protein precipitation extraction using a validated LC-MS/MS assay. Calibration standard responses were linear over the 5.00 to 1,000 ng/mL using a weighted (1/concentration²) linear regression. The lower limit of quantification was 5.00 ng/mL. The plasma concentrations of MTX were analyzed following a non-compartmental approach (WinNonLin®, version 5.3; Pharsight corporation, Mountain View, CA, USA). The following parameters were determined: Cmax, tmax, AUC0-24h, and the area under the curve up to the last sampling time point, i.e., 8 h (AUC0-8h).

Potential drug-drug interaction between filgotinib and MTX was assessed using ln-transformed MTX parameters (Cmax, AUC0-8h) by means of a mixed effect ANOVA including treatment (MTX alone or MTX + filgotinib), filgotinib dose group (excluding placebo) and the interaction between filgotinib dose group and the treatment as fixed effects, and subject nested with the filgotinib dose group as a random effect. Point estimates were calculated for each parameter as the geometric mean ratio of MTX+filgotinib relative to day-1 (MTX) with corresponding 90%CI. The interaction of filgotinib on MTX pharmacokinetics was assessed for all filgotinib dose groups together as well as for each filgotinib dose group separately. Although the study was not statistically powered, the lack of an interaction on MTX pharmacokinetics would be demonstrated if the 90%CI for the ratio of adjusted geometric means for Cmax and AUC0-8h fell wholly within the 80-125% interval. SAS® version 9.1 (SAS institute Inc. Cary, NC, USA) at the
0.05 level of significance was used for statistical inferential analyses.

RESULTS

Identification of the Enzymes Involved in Filgotinib Metabolism

The investigation of the metabolism in vitro revealed the presence of one major metabolite in all the species tested (rat, mouse, monkey, dog, and human; data not shown). This metabolite has been structurally identified and resulted from the loss of the cyclopropyl carboxylic acid group (Fig. 1).

The type of enzymes involved in filgotinib’s metabolism was first characterized by monitoring the formation of the major metabolite by human liver microsomes in presence or absence of NADPH. The results of this first experiment showed that the amount of metabolite formed, expressed as percentage of filgotinib, was similar with or without the co-factor [mean (SD): 4.40% (0.426%) vs 4.95% (1.17%)]. Furthermore, the metabolism was completely inhibited in presence of bis-pNPP. These data suggest the metabolism of filgotinib to be non-CYP450-dependent and to be mediated by hydrolases. Given the structure of the metabolite (Fig. 1), hydrolases such as carboxylesterases (CES) were envisaged as potential enzymes responsible for its formation. The results of the second experiment carried out using human recombinant enzymes demonstrated that filgotinib is mainly metabolized by hCES2 with about 70% of the total amount formed by recombinant enzymes (Table 1). At the highest concentration tested (60 µM) a clear saturation of hCES2 was observed (25.7% of total amount of metabolite formed) while both hCES1 produced the metabolite with 6.32%, and 6.12% of total amount of metabolite formed by hCES1b, and hCES1c, respectively.

Experimental in vitro Systems to Assess CYP450 Inhibition

The inhibition of human CYP450 enzymes by filgotinib and its metabolite was evaluated using HLMs and selective probe substrates. At the highest concentration tested (70 µM), filgotinib weakly inhibited CYP1A2, CYP2A6, and CYP3A4 (testosterone, only) with a percentage of inhibition of 21-24%, which is insufficient to calculate an IC_{50}. Similarly for the metabolite, marginal inhibition was noted on CYP2A6. The maximum inhibition (50-61%) was observed for CYP2C9 and CYP2C19 at the highest concentration of metabolite tested, which corresponds approximately to an IC_{50} value of 224 µM. At clinically relevant concentrations (i.e., 5- to 7-fold the C_{max} of filgotinib and its active metabolite observed after a 200 mg once daily dose of filgotinib, which is the highest dose tested in Phase 2b dose-finding studies in RA patients), there was no meaningful inhibition of any CYP450 for both filgotinib and its metabolite (Fig. 3).

Experimental in vitro System to Assess CYP450 Induction

The potential for filgotinib and its metabolite to induce CYP1A2, CYP2B6, and CYP3A4 was assessed in human hepaRG® cell lines using reference substrates to assess changes in CYP450 activity and determine the induction for each CYP450. After incubation for 48 hours with filgotinib or its metabolite, there was no notable induction of CYP1A2, CYP2B6, and CYP3A4 at concentrations above 2-fold the C_{max} value observed at a 200 mg daily dose of filgotinib (Table 1).

Experimental in vitro System to Assess UGT Inhibition

The inhibition of human UGT enzymes by filgotinib and its metabolite was measured using recombinant enzymes and selective probe substrates. At the highest concentration tested (60 µM), filgotinib weakly inhibited UGT1A9 (22.4%) and UGT1A1 (31.0%) (Table 2). Similarly for the metabolite, marginal inhibition was noted for UGT1A6 (23.2%), UGT1A9 (21.7%), and UGT2B7 (37.8%). Based on these results, the IC_{50} value for each of the five UGTs is higher than 60 and 80 µM for filgotinib and its metabolite, respectively.

Experimental in vitro System to Assess Drug Transporters Inhibition

The inhibition of P-gp (MDR1) and BCRP by filgotinib and its major metabolite was measured by determining the A-B and B-A transports of 3H-digoxin (5 µM) and 3H-prasozin (1 µM) across the MDCKII-MDR1 and MDCKII-BCRP monolayer cells, respectively. Transports in presence of specific inhibitors, PSC833 (10 µM) for P-gp and Ko134 (1µM) for BCRP, were also included to validate the experiment. At 50 µM, none of the two compounds inhibited significantly the P-gp-mediated transport of 3H-digoxin on MDCKII-MDR1 monolayer cells with a decrease by 34-37% of the digoxin efflux ratio (Fig. 4 Panel A). Similarly the BCRP-mediated transport of 3H-prasozin on MDCKII-BCRP monolayer cells was unaffected by the presence of 50 µM of filgotinib or its metabolite. These data suggest that the IC_{50}
values for both efflux transporters would be much higher than 50 µM for both compounds.

The inhibition of BSEP by filgotinib and its metabolite was assessed by measuring the transport of the probe substrate, ³H-taurocholate (2 µM), across membrane vesicles over-expressing the human transporter. A specific inhibitor (cyclosporine A, 20 µM) was also included as positive control. As shown in Fig. 4 (Panel B), neither filgotinib nor its metabolite inhibited BSEP-mediated transport of ³H-taurocholate, suggesting the IC₅₀ value for this transporter to be higher than 60 µM for both compounds.

The inhibition of OATs by filgotinib was investigated using the Human Embryonic Kidney (HEK) MSRII cell line expressing the human OAT1, OAT3, and OAT4. Inhibition
of the uptake of the probe substrate (6-carboxyfluorescein, 6-CFL, 5-20 µM) was monitored in presence of filgotinib concentrations (up to 100 µM) or specific inhibitor (benzbromarone, 30-60 µM). For the metabolite, the potential for inhibition of OAT1 and OAT3 was investigated using different cell lines over-expressing the human transporter: OAT1-CHO, OAT3-HEK293. In this second experiment, the inhibition of uptake of probe substrates (3H-p-aminohippuric acid 0.5 µM for OAT1 and 3H-estrone-3-sulfate 0.2 µM for OAT3) was followed in presence of the metabolite (up to 300 µM) or specific inhibitors (benzbromarone 200 µM for OAT1 and probenicid 200 µM for OAT3). As shown in Fig. 4 (Panel B), neither filgotinib nor its metabolite showed marked interaction with OATs at a concentration of at least 5 times the Cmax values obtained at 200 mg daily in human. The maximum inhibition (58%) was observed for OAT3-substrate-mediated transport with filgotinib, suggesting the IC50 value for this transporter to be approximately 100 µM.

The inhibition of OATPBs and OCTs by filgotinib and its metabolite was investigated using CHO cell lines over-expressing each of the human OATP1B1, OATP1B3, OCT1, or OCT2 transporters. Specific inhibitors (cerivastatin 100 µM for OATP1B1; fluvastatine 30 µM for OATP1B3; verapamil 100 µM for OCT1 and cimetidine 1 mM for OCT2) were also included as positive controls. At a concentration of at least 5 times the Cmax at a 200 mg daily dose of filgotinib, the uptake of OATP1B1 (3H-estrone-3-sulfate, 0.1 µM) and OATP1B3 (Fluo-3, 10 µM) substrates was not impacted by filgotinib or its metabolite (Fig. 4 Panel B). The substrate-mediated transport was inhibited by 10-20% for OCT1 (14C-tetraethylamine chloride, 3.6 µM) and by 40-60% for OCT2 (14C-metformin, 2 µM). These data suggested the IC50 values for these transporters to be higher than 60 µM for both compounds, except for OCT2 where calculated IC50 values were 8.7 and 67 µM for filgotinib and its metabolite, respectively.

### Table 2. Inhibition of UGTs by filgotinib and its major metabolite using human recombinant enzymes.

<table>
<thead>
<tr>
<th>UGT</th>
<th>Probe Substrate</th>
<th>Reference Inhibitor</th>
<th>IC50 (µM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>β-estradiol (20 µM)</td>
<td>Ketoconazole (100 µM)</td>
<td>&gt;60</td>
<td>&gt;80</td>
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<td>Hecogenine (10 µM)</td>
<td>&gt;60</td>
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<td>Deferiprone (1 mM)</td>
<td>Troglitazone (100 µM)</td>
<td>&gt;60</td>
<td>&gt;80</td>
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<td>Zidovudine (70 µM)</td>
<td>Fluconazole (50 mM)</td>
<td>&gt;60</td>
<td>&gt;80</td>
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hUGTs (0.1-0.2 mg/mL) buffer, filgotinib or its metabolite pre-incubated for 15 minutes with UDPGA prior to initiation of reaction with probe substrates. Experiments conducted in triplicate.

NIO: no relevant inhibition observed (<20% inhibition).

* % inhibition at the highest concentration tested (60µM for filgotinib and 80µM for its metabolite).
Drug-drug Interaction with Midazolam in Healthy Volunteers

Twenty male subjects aged between 40 and 60 years were enrolled in the study. Once daily oral doses of filgotinib co-administered with midazolam were considered safe and well-tolerated. No severe or serious adverse events were reported and there were no clinically significant changes in vital signs, clinical laboratory parameters, and ECG findings.

The mean plasma midazolam concentration profiles in the absence of filgotinib or after a once daily dose of 200 mg filgotinib were similar (Fig. 5).

Statistical analysis comparing midazolam alone or in combination with filgotinib showed that the geometric mean ratio (90% CI; midazolam + filgotinib / midazolam alone) for $C_{\text{max}}$, $\text{AUC}_{0-\text{t}}$, $\text{AUC}_{0-\infty}$, and $t_{1/2,\text{z}}$ were all within the 80% to 125% range. The results indicate that once daily 200 mg doses of filgotinib did not affect the pharmacokinetics of the single 2 mg dose of midazolam in healthy male subjects (Table 3).

Drug-drug Interaction with Methotrexate in RA Patients

RA patients from the short term efficacy study who participated in the MTX pharmacokinetic sample collection were dosed with 7.5 mg/kg to 20 mg/kg weekly MTX doses [15]. Due to the low number of patients per filgotinib dose group (N=3 to 5), the potential for drug-drug interaction of filgotinib on MTX pharmacokinetics was assessed by pooling data from all filgotinib dose groups (N=17). Results of the statistical analysis showed that both $C_{\text{max}}$ and $\text{AUC}_{0-8h}$

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\text{Table 3. Effect of once daily dose filgotinib on the single dose pharmacokinetics of midazolam.}
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<table>
<thead>
<tr>
<th></th>
<th>Midazolam (2 mg)</th>
<th>Midazolam (2 mg) + Filgotinib (200 mg q.d.)</th>
<th>PE (90%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>8.70 (30.6)</td>
<td>8.82 (37.0)</td>
<td>99.9% (87.6% - 112.5%)</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>0.5 (0.3-1.0)</td>
<td>0.5 (0.5-1.0)</td>
<td>NA</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\text{t}}$ (ng.h/mL)</td>
<td>20.7 (34.1)</td>
<td>21.6 (45.0)</td>
<td>100.3% (89.9% - 111.9%)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (ng.h/mL)</td>
<td>21.9 (34.9)$^{\text{N=19}}$</td>
<td>24.0 (39.5)$^{\text{N=19}}$</td>
<td>105.4% (97.8% - 117.3%)</td>
</tr>
<tr>
<td>$t_{1/2,\text{z}}$ (h)</td>
<td>3.29 (29.9)$^{\text{N=19}}$</td>
<td>3.64 (28.6)$^{\text{N=18}}$</td>
<td>108.8% (97.0% - 121.9%)</td>
</tr>
</tbody>
</table>

Estimates are expressed as arithmetic means (CV%) except median(min-max) for $t_{\text{max}}$; N=20
NA: not applicable
$C_{\text{max}}$: maximal concentration; $t_{\text{max}}$: time to reach maximal concentration; $\text{AUC}_{0-\text{t}}$: AUC up to the last quantifiable concentration; $\text{AUC}_{0-\infty}$: AUC extrapolated up to infinity; $t_{1/2,\text{z}}$: apparent terminal elimination half-life.
PE (point estimate): pairwise comparison between the test (midazolam + filgotinib) and the reference (midazolam) treatment with its corresponding 90% confidence interval (CI).
of MTX were not impacted by the co-administration with filgotinib up to a 300 mg daily dose (Table 4).

Although this investigation was not powered to assess the potential of interaction using the 90% CI approach, the interval boundaries for both C\text{max} and AUC\text{0-8h} of MTX were close to, or even fell within, the 80–125% bioequivalence range: 98.76% (85.43%-114.17%) and 102.72% (84.02%-125.58%), respectively.

**DISCUSSION**

The *in vitro* characterization of the main enzyme(s) involved in the metabolism of filgotinib revealed that carboxylesterases (CES) are responsible for the formation of an active metabolite which is also the major one in humans. These enzymes, members of the α/β-hydrolase family, are abundant and display ubiquitous tissue expression profiles [16]. The majority of CES belongs to the CES1 and CES2 families. Human CES2 enzymes are localized mainly in the intestine and to a lesser extent in the liver, while CES1 family enzymes are more prominent in the liver. These two CES families show differences in substrate specificity: hCES2 has limited ability to hydrolyse compounds that contain a large acyl moiety, whereas hCES1 can hydrolyse a wide variety of substrates [16-19]. From the *in vitro* experiment using hCES recombinant enzymes, it has been shown that hCES2 is the main isoform responsible of the formation of its major, active metabolite. These results are consistent with the data reported on CES substrate specificity.

Of interest, some saturation of hCES2 was noticed *in vitro* at high filgotinib concentrations while both hCES1 isoforms formed the active metabolite in similar proportion. These data demonstrate that even in case of complete inhibition and/or saturation of CES2, the metabolic elimination of filgotinib would not be completely impaired and liver CES1 could also form the active metabolite.

Results from *in vitro* investigations showed that neither filgotinib nor its active metabolite induce nor inhibit CYP450s at concentrations of at least 2 to 5-fold the C\text{max} value following daily dosing of 200 mg filgotinib, which is the highest dose selected for Phase 2B dose-finding studies in RA patients [4]. To confirm the conclusions from these *in vitro* data, the potential for interaction with CYP3A4 was investigated in healthy volunteers using midazolam (2mg) administered alone or after filgotinib (200 mg) once daily dosing for 7 days. Results of this study demonstrated that the pharmacokinetics of midazolam were not impacted by daily administration of filgotinib. These clinical data confirmed and validated the *in vitro* results. As no interaction was noted *in vitro* for all the other CYPs as well, a broader conclusion on the lack of interaction of both filgotinib and its active metabolite on CYPs could be made.

Subsequent metabolism by CES and UGTs in the luminal sides of the endoplasmic reticulum membrane has been previously reported [18]. An investigation of a potential interplay between CES and UGT in the metabolic pathway of filgotinib confirmed the formation of the N-glucuronide of the main metabolite (data not shown; Fig. 1). Therefore, the potential of filgotinib and its main active metabolite to inhibit liver UGTs was performed and revealed no significant inhibition of UGTs suggesting the risk of inhibition of UGTs in a clinical setting to be low. Consistent with this, no clinical elevations in bilirubin, a substrate of UGT1A1, were observed during once daily administration up to 450 mg filgotinib to healthy subjects [20].

Several papers reported the interaction of gemfibrozil 1-O-β-glucuronide, with CYP2C8 substrates such as cerivastatin [21] and rosiglitazone [22]. This glucuronide is an acyl glucuronide involved in covalent binding reactions and irreversible inactivation of CYP2C8 [23]. Although it could not be completely rule out no interaction with CYP450s have been published so far with N-glucuronides. In addition, glucuronidation is a predominant pathway in
gemfibrozil metabolism while for filgotinib, the plasma exposure to N-glucuronide metabolite derivate is low and represents 11% and 2.7% of filgotinib and active metabolite exposures. Consequently the risk for clinically relevant interaction of the N-glucuronide metabolite derivate is unlikely.

The pharmacokinetics of filgotinib and of its major active metabolite appeared to be dose linear up to 200 mg daily dose [15, 20]. These data suggest that, within the therapeutic dose range, the transporters potentially playing a role in filgotinib disposition, if any, were not saturated or induced. The potential inhibition of drug transporters by filgotinib or its active metabolite was investigated in vitro. The IC₅₀ values for efflux transporters (P-gp and BCRP) were well above 50 μM and thus at least 15- and 5-fold higher than the Cmax values for filgotinib and its active metabolite, respectively, following daily doses of 200 mg filgotinib. Due to limitations in solubility, the IC₅₀ for filgotinib could not be determined. Therefore the inhibition of these two transporters using estimated intestinal concentrations following a 200 mg filgotinib dose was not assessed. Besides OCT2, the IC₅₀ values for all transporters examined were 60 μM or higher, being at least 18- and 6-fold higher than the Cmax values for filgotinib and its active metabolite, respectively, at 200 mg filgotinib. The clinical relevance of the observed isolated in vitro inhibition of OCT2 is difficult to judge given that many drugs are substrates of multiple transporters.

With the aim to have clinical confirmation of the lack of a relevant inhibition, the potential for interaction with OATs, the renal transporters involved in elimination of MTX [24], was investigated in an exploratory dose ranging Phase 2A study in RA patients who received filgotinib and MTX concomitantly [15]. Results of this study demonstrated that the daily administration of filgotinib up to 300 mg did not influence the pharmacokinetics of MTX. These clinical data confirm the lack of interaction of both filgotinib and its active metabolite on these transporters eliminating MTX.

No validated in vitro systems to assess transporter induction are available yet. Nevertheless, as the induction of both P-gp and CYP3A4 is regulated by the pregnant X receptor, it could be concluded that at least induction of P-gp is unlikely to occur after repeated dosing with filgotinib.

CONCLUSION

In vitro, filgotinib and its active metabolite do not interact with CYP450s and do not inhibit UGTs and key drug transporters at clinically relevant concentrations, with a possible exception of OCT2. In vitro data have been confirmed by clinical data using midazolam as a probe drug for CYP3A4 and MTX as a probe for elimination through drug transporters (OATs). The lack of relevant drug interactions with CYP450 substrates by filgotinib or its metabolites, either through inhibition or induction, support the use of filgotinib with concomitant drugs without the need for dose adjustment of filgotinib or of concomitant medications.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.
inhibitor, after short-term treatment of rheumatoid arthritis; results of two Phase IIA trial; data on file.


