Dicloxacillin induces CYP2C19, CYP2C9 and CYP3A4 in vivo and in vitro

Short title: Dicloxacillin induces CYP2C- and CYP3A-mediated drug metabolism

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Summary

Aim

The aim of this study was to study potential cytochrome P450 induction by dicloxacillin.

Methods

We performed an open-label randomized two-phase 5-drug clinical pharmacokinetic cocktail crossover study in 12 healthy men with and without pretreatment with 1g dicloxacillin three times daily for 10 days. Plasma and urine was collected over 24 hours and the concentration of all five drugs and their primary metabolites was determined using a LC-MS/MS method. Cryopreserved primary human hepatocytes were exposed to dicloxacillin for 48h and changes in gene expression and enzyme activity of CYP3A4, CYP2C9, CYP2B6 and CYP1A2 was investigated. Activation of nuclear receptors by dicloxacillin was assessed using luciferase assays.

Results

Ten days of treatment with dicloxacillin resulted in a clinically and statistically significant reduction in the area under the plasma concentration-time curve from 0-24h of omeprazole (CYP2C19) (geometric mean ratio (GMR) [95% confidence interval (CI)]: 0.33 [0.24-0.45]), tolbutamide (CYP2C9) (GMR [95% CI]: 0.73 [0.65-0.81]) and midazolam (CYP3A4) (GMR [95% CI]: 0.54 [0.41-0.72]). Additionally, other relevant pharmacokinetic parameters were affected indicating induction of CYP2C and CYP3A4-mediated metabolism by dicloxacillin. Investigations in primary hepatocytes showed statistically significant dose-dependent increase in P450 expression and activity by dicloxacillin, caused by activation of pregnane X receptor.
Conclusions

Dicloxacillin is an inducer of CYP2C- and CYP3A-mediated drug metabolism and we recommend caution when prescribing dicloxacillin to users of drugs with a narrow therapeutic window.

What is already known about this subject?

- Dicloxacillin is known to reduce the therapeutic efficacy of warfarin treatment, but the mechanism is unknown.

What this study adds

- This study shows that dicloxacillin is a clinically relevant inducer of CYP2C9-, CYP2C19- and CYP3A4-mediated expression and activity. In vitro results show that this is caused by PXR activation leading to increased expression and activity of these enzymes and clinicians prescribing dicloxacillin should be aware that dicloxacillin induces CYP2C9-, CYP2C19- and CYP3A4-mediated metabolism.
Introduction

Dicloxacillin belongs to the group of narrow-spectrum isoxazolyl beta-lactam penicillins (Figure 1) and is primarily used for skin-, soft tissue-, or bone infections caused by Staphylococcus aureus infection [1]. As dicloxacillin exhibits strong time-dependent antibacterial activity, the time above minimal inhibitory concentration (0.125 µg/ml) is crucial for its clinical effect [2]. Due to a short elimination half-life of dicloxacillin (60-90 minutes after oral ingestion [3,4]), administration every 6-8 hours is necessary to sustain sufficient anti-bactericidal concentrations. Following 500 mg four times daily of orally administered dicloxacillin, plasma concentrations fluctuate from 2 µM (C_min) to 57 µM (C_max) [4]. Data on the utilization patterns of beta-lactamase resistant penicillins in general, and dicloxacillin specifically, is scarce [5]. Dicloxacillin is the dominant choice of isoxazolyl penicillins in both Denmark and Norway, with annual prevalences of 25 [6] and 20 (www.reseptregisteret.no) per 1000 inhabitants, respectively.

In humans dicloxacillin is primarily excreted unchanged renally, but also metabolized to some extent to the 5-hydroxy metabolite [7], though it is unclear which P450 enzymes are involved in the metabolism. Dicloxacillin is a substrate of P-glycoprotein (P-gp, ABCB1) [8] and concomitant intake of rifampicin leads to lower plasma concentrations of dicloxacillin, accompanied by increased plasma concentrations of the 5-hydroxy metabolite. This is likely caused by a combination of induction of P450 enzymes relevant for dicloxacillin metabolism and induction of intestinal P-gp [9].

Several case reports have described that initiation of either dicloxacillin or cloxacillin treatment leads to decreased international normalised ratio (INR), a proxy biomarker of clinical efficacy, during treatment with the vitamin K antagonist warfarin [10–13]. In a recent
observational study of 236 patients, we confirmed that initiation of dicloxacillin resulted in markedly decreased INR values during warfarin treatment [14], leading to sub-therapeutic INR levels in 60% of the treated patients. Additional case reports have described that initiation of treatment with another isoxazolyl penicillin, flucloxacillin, resulted in decreased plasma levels of voriconazole [15] and quinidine [16]. The mechanistic basis for drug-drug interactions with isoxazolyl penicillins is unknown. Sparse in vitro data have indicated that dicloxacillin activates *pregnane X receptor* (PXR) causing increased CYP3A4-mediated testosterone metabolism in human hepatocytes [17] and induction of CYP2C9 [18]. PXR activation leading to upregulation of P450 enzymes and subsequent increased metabolism may provide an explanation for the abovementioned drug-drug interactions, but this has not been confirmed in clinical pharmacokinetic studies.

The objective of this study was to determine the effect of treatment with a clinically relevant treatment course of dicloxacillin on the metabolic capacity of CYP3A4, CYP2C9, CYP2C19, CYP1A2 and CYP2D6 in healthy volunteers. We investigated the underlying mechanism *in vitro* using cryopreserved human hepatocytes to assess changes in expression and activity of P450 enzymes and nuclear receptor activation by dicloxacillin.
Methods

Clinical study

This study was designed as an open-label randomized two-phase 5-drug clinical pharmacokinetic cocktail study to assess the potential of dicloxacillin to induce P450 enzymes. We used a validated and previously used 5-drug cocktail [19–21] to assess metabolic capacity of major drug metabolizing enzymes (CYP2C9: Tolbutamide, CYP3A4: Midazolam, CYP2C19: Omeprazole, CYP1A2: Caffeine, and CYP2D6: Dextromethorphan).

Pharmacokinetic cocktail studies are used to assess the metabolic capacity of CYP3A4, CYP2C19, CYP2D6 and CYP1A2 simultaneously by pharmacokinetic assessment of P450-specific probes. We used a modified Cooperstown cocktail (midazolam, omeprazole, dextromethorphan and caffeine) [22]. Since (S)-warfarin (the most pharmacologically active isomer of racemic warfarin) is metabolized by CYP2C9, we added tolbutamide to the cocktail, a well-validated substrate and biomarker for CYP2C9 metabolism [23]. This combination of biomarker drugs has previously been validated [19] and used to evaluate changes in drug metabolism caused by drug-drug interactions [20,21].

Study medication and dose

Tolbutamide (Arcosal® one 500 mg tablet, Meda AS, Allerød, Denmark), dextromethorphan (Dexofan® one 30 mg tablet, Takeda Pharma, Taastrup, Denmark), omeprazole (Omestad® one 20 mg enteric capsule, Stada Nordic ApS, Herlev, Denmark) and caffeine (two 100 mg caffeine tablets produced at Glostrup Hospital Pharmacy, Copenhagen, Denmark) were all administered orally. Midazolam was administered as a buccal solution of 2.5 mg (Buccolam®, Shire Sweden AB, Stockholm, Sweden) between the cheek and the gums slowly at least 3 minutes after the oral drugs were ingested. Dicloxacillin was administered as
two 500 mg capsules (Dicloxacillin “Alternova”®, Alternova A/S, Odense, Denmark) three times daily for 10 days; at least one hour before or at least two hours after a meal. This is the dicloxacillin dosage regimen for treatment of Staphylococcus aureus in Denmark, as recommended by the Danish Physicians’ Desk Reference (www.pro.medicin.dk).

Design and study criteria

Twelve healthy men were recruited for this two-phase study and ingested the 5-drug cocktail in both phases after 12 hours fasting. Women were excluded due to a putative clinically relevant drug-drug interaction between oral contraceptives and dicloxacillin. In phase A, no concomitant drugs were used. In phase B, dicloxacillin was administered as 1 g three times daily for 10 days prior to the study day. On the morning of day 11, the 5-drug cocktail was ingested as in phase A. There was a washout period of at least six weeks between the two phases (Figure 2). Plasma samples were drawn at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours. Urine was collected from 0-12 h and 12-24 h for determination of metabolites. Three hours after ingesting the drugs the participants were given a meal. Inclusion criteria for this study were: Non-smoking healthy men aged 18-55 years, body mass index (BMI) within 18.5-29.9 kg/m² and estimated glomerular filtration rate (eGFR), alanine transaminase (ALAT), bilirubin, haemoglobin and glycosylated haemoglobin A1c (HbA1c) within the reference range. Exclusion criteria for this study were: Known allergies to any of the used drugs, known penicillin allergy or type I reaction to cephalosporines, known allergy to sulphonylureas, intake of prescription drugs, over-the-counter drugs, herbal medicines or supplements known to affect drug pharmacokinetics, chronic or daily alcohol abuse and participation in other intervention trials. Healthy status and use of drugs were assessed by answers to the following five questions: Are you healthy? Do you suffer from any chronic diseases? Do you ingest drugs on a daily basis? Do you occasionally use prescription drugs?
Do you use any over-the-counter or herbal medicines or supplements? A data manager performed block-randomisation using Sealed envelope, which is freely available at www.sealedenvelope.com. The list was then made accessible to trial investigators using RedCap [24].

**Study approvals**

The clinical study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice (GCP) and monitored by the GCP Unit, Odense University Hospital, Odense, Denmark. The study protocol was approved by the Danish Medicines Agency (identifier 2016043478), registered in the EudraCT database (identifier 2016-001334-10) and the Regional Scientific Ethical Committee of Southern Denmark (identifier S-20160073) and all subjects consented to participate in the study. The trial was registered at http://www.clinicaltrials.gov (identifier NCT02983890).

**Determination of drugs and their metabolites in plasma and urine**

The analysis for the five probe drugs for major P450 isoenzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were performed in plasma and urine samples. The samples were analysed for caffeine, paraxanthine, omeprazole, 5-hydroxyomeprazole, tobutamide, 4-hydroxytolbutamide, dextromethorphan, dextrorphan, midazolam and α-hydroxymidazolam. The analysis of plasma was performed according to the method described by Wohlfahrt et al. [19] with minor modification, using isotope dilution, solid-phase extraction (SPE) and liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS). Urine samples were deglucuronidated prior to addition of the isotope-labelled internal standard and dilution with mobile phase before injection onto the LC-MS/MS. Analysis of omeprazole and its metabolite in urine was performed prior to the
deglucuronidation procedure as these compounds are not stable during this acidic process.

The LC-MS/MS system consisted of a Dionex Ultimate 3000 UHPLC system equipped with an EQUAN autosampler unit; a Dionex Ultimate 3000 RS column compartment, an Ultimate 3000 RS Pump, connected to a TSQ Quantiva Triple Quadropole Mass Spectrometer, with heated-electrospray ionization operated in positive mode (Thermo Scientific, San José, CA). Data acquisition was performed in single reaction monitoring (SRM) mode, with transitions optimized for all the compounds. Calibration curves, blanks as well as quality control samples were included in each batch of samples analysed. The method was linear with $r > 0.9992$. The between-day reproducibility and intra-day repeatability was <5.2% and <5.5%, respectively, for all compounds. The accuracy reported as bias ranged from -2.6 – 1.8%. The LOD ranged from 0.05 – 0.75 ng/mL and LOQ ranged from 0.1 – 1.5 ng/mL [19].

**Genotyping**

Briefly, a Maxwell™ 16 Blood DNA kit was used to isolate DNA from whole blood samples per manufacturer’s protocol. Single nucleotide polymorphisms (CYP2C9*2/*3 and CYP2C19*2/*3/*17 and CYP2D6*3/*4/*6/copy number variation (*N), (Supplementary Table 1) were genotyped using predesigned TaqMan SNP genotyping assays on a StepOne Plus real-time instrument (Applied Biosystems, Foster City, CA, USA) per manufacturers’ protocol.

**Statistics and pharmacokinetic analysis**

The primary endpoint in this study was difference in tolbutamide AUC with and without dicloxacillin. To detect a difference of ≥25% in tolbutamide AUC$_{0-24h}$ with 80% power, a two-sided significance level of 5% and allowing a dropout rate of 20%, a total of 12 healthy
individuals were required for this study. Demographic data are shown as medians with interquartile ranges (IQR, 25th–75th percentiles). Pharmacokinetic endpoints are presented as medians with IQR and geometric mean ratios with 95% confidence intervals. Statistical significance was determined using paired t-test and accepted at p-values < 0.05.

Pharmacokinetic data were analysed by non-compartmental analysis using the R package NCAPPC [25]. Area under the plasma concentration-time curve was estimated using the linear up logarithmic down method. CLf and CLR were estimated using the following equations:

\[
\text{CL}_f = \frac{\text{Amount of metabolite in urine}_{0-1}}{\text{AUC of substrate}_{0-1}}
\]

\[
\text{CL}_R = \frac{\text{Amount of substrate in urine}_{0-1}}{\text{AUC of substrate}_{0-1}}
\]

Due to short elimination half-lives of omeprazole and midazolam, 0-12 h formation and renal clearances were calculated, while 0-24 h values were calculated for caffeine, dextromethorphan and tolbutamide.

**In vitro**

**Cell culture**

The methods for cell culture, gene expression and CYP activities in human hepatocytes were described in detail previously [26]. Briefly, cryopreserved human hepatocytes (male lots: HU1765 (ThermoFisher catalog #HMCPI), HUM4034 (Triangle Research Labs catalog #HUCPI), female lots: HH1057 (In Vitro ADMET Laboratories catalog #82006), OII (Bioreclamation IVT catalog #F00995-P)) were plated at 70,000 cells/well in collagen-coated 96-well plates in CHRM™ medium to obtain a monolayer of cells. After 4 hours incubation, the cells were overlaid with cold incubation media containing 0.35 mg/ml GelTrex®. The
following day, the medium was replaced with fresh medium and incubated for another 24 hours. On day three, the cells were incubated for 48 hours with dicloxacillin, flucloxacillin, rifampicin (PXR agonist, CYP3A and CYP2C regulation), phenobarbital (constitutive androstane receptor (CAR) agonist, CYP2B6 regulation) or 3-methylcholanthrene (aryl hydrocarbon receptor (AhR) agonist, CYP1A regulation) in increasing concentrations or with vehicle (0.1% DMSO).

**Gene expression and activity of P450 enzymes**

Changes in gene expression for a panel of drug metabolizing enzymes and several transporters were determined using a QuantiGene Plex 2.0 assay kit. Activity of relevant P450 enzymes was assessed using well-validated probe drugs (CYP3A4: 100 µM testosterone, CYP2C9: 100 µM diclofenac, CYP2B6: 500 µM bupropion and CYP1A2: 100 µM phenacetin). After incubation with dicloxacillin, flucloxacillin and positive controls, hepatocytes were incubated with probe drugs for 90 minutes and metabolites were measured using an established LC-MS/MS method [27]. Activity and gene expression values are presented as activity/expression relative to vehicle (0.1% DMSO).

**Nuclear receptor activity**

Activation of PXR, CAR and AhR was assessed by Puracyp laboratories (Carlsbad, CA, USA) as previously described [28,29]. Briefly, a HepG2-derived cell line stably transfected with nuclear receptors and corresponding response elements were seeded in 96-well plates. Twenty-four hours after seeding, the cells were exposed to six different concentrations of dicloxacillin and flucloxacillin and incubated another 24 hours. Receptor activity was assessed using Promega’s ONE-Glo® luciferase assays and cytotoxicity was assessed using Promega’s Cell Titer Flour® assay. Positive controls for PXR, CAR and AhR activation were
rifampicin, CITCO and 3-MC, respectively. Data for nuclear receptor activity are adjusted for viability and expressed relative to vehicle control.
Results

Clinical study

This study was an open two-phase randomised clinical pharmacokinetic crossover study in 12 healthy men (Figure 2). The median age was 22 years (interquartile range (IQR): 21-24, range 21-29) and median body mass index was 24.3 kg/m² (IQR: 23.3-25.6, range: 20.4-28.7). A 5-drug cocktail of midazolam (CYP3A4) (2.5 mg buccal), tolbutamide (CYP2C9) (500 mg tablet), omeprazole (CYP2C19) (20 mg enteric capsule), caffeine (CYP1A2) (2x100 mg tablets) and dextromethorphan (CYP2D6) (30 mg tablet) was administered before and after 10 days treatment with 1 g dicloxacillin three times daily. The sequence of study phases was randomised to avoid period effects and a washout period of at least 6 weeks was required between the two phases to avoid carryover effects. Two subjects suffered nausea and dizziness following multiple attempts at insertion of the cubital vein cannula. Following treatment with dicloxacillin, one subject described dyspepsia and stomach pain, and one subject had an erythematous rash on the extremities, both well-described adverse reactions [30]. All adverse reactions were transient and not deemed serious.

Pharmacogenetics

Subjects were genotyped for the most common functional genetic variants in CYP2D6 (*3; *4; *6 and copy number variation *N), CYP2C9 (*2; *3) and CYP2C19 (*2; *3; *17) after conclusion of the study. Genotypes for the 12 subjects are shown in Supplementary Table 1. One CYP2D6 poor metaboliser, two CYP2C19 ultrarapid metabolisers and two CYP2C9 poor metabolisers were included in the study. The main analysis presented in this paper is intention to treat, including all subjects regardless of genotype. A sensitivity analysis excluding individuals carrying altered function variants did not affect the conclusions of the study (Supplementary Table 2).
**Pharmacokinetics**

Area under the concentration-time curve from 0-24h (AUC\(_{0-24h}\)), maximum plasma concentration (C\(_{\text{max}}\)), elimination half-life (T\(_{\frac{1}{2}}\)) and formation clearance (CL\(_{f}\)) for the main metabolite of all five probe drugs with and without dicloxacillin exposure are shown in Table 1 and concentration-time curves for the five probes with and without dicloxacillin are depicted in Figure 3. Individual CL\(_{f}\) with and without dicloxacillin exposure is shown in Figure 4. Detailed pharmacokinetic results are shown in Supplementary Table 3, which contains pharmacokinetic data for all probe drugs and their metabolites. Full pharmacokinetic profiles were available for all 12 individuals for all drugs except caffeine; two subjects were excluded from the caffeine analysis since they violated the protocol by ingesting caffeine during the trial.

With concomitant dicloxacillin treatment, the AUC of all probe drugs was significantly reduced, without changes in renal clearance (Table 1 & Supplementary Table 3, Figure 3). Tolbutamide AUC and C\(_{\text{max}}\) were reduced by 27% and 7% respectively, driven by increased formation of the 4-OH tolbutamide metabolite as reflected in a 64% increase in CL\(_{f}\) of the metabolite (Figure 4). Omeprazole AUC and C\(_{\text{max}}\) were 67% and 60% lower with dicloxacillin exposure. Although the CL\(_{f}\) of the CYP2C19-mediated 5-OH omeprazole metabolite was twice as high with dicloxacillin exposure, this was not statistically significant (p=0.18, geometric mean ratio (GMR) [95% confidence interval]: 2.01 [0.69-5.89]).

Dicloxacillin treatment also led to 46% reduction in midazolam AUC, attributed to increased CYP3A4 metabolism as reflected by a 59% increase in CL\(_{f}\) of the \(\alpha\)-hydroxymidazolam metabolite. Ratio of midazolam to metabolite AUC increased slightly with significant imprecision (GMR [95% CI]: 1.27 [0.89-1.80]). Dicloxacillin also caused minor changes in
caffeine pharmacokinetics; caffeine AUC was 19% lower with dicloxacillin with no change in CL, suggesting the changes in AUC were not caused by increased CYP1A2-mediated paraxanthine metabolism. Dicloxacillin also caused a marked 48% decrease in dextromethorphan AUC.

**Human hepatocytes**

The effect of dicloxacillin on expression and activity of CYP3A4, CYP2C9, CYP2B6 and CYP1A2 was assessed in cryopreserved hepatocytes from four donors (2 males and 2 females). Data from all donors is shown in Figure 5 and individual data from the four donors are shown in Supplementary Figures 1-4. All data are shown relative to 0.1% DMSO control and all mentioned results were statistically significant. Dicloxacillin led to dose-dependent upregulation of CYP3A4, CYP2C9 and CYP2B6, but not CYP1A2 expression, in all hepatocyte samples (Figure 5, top). CYP3A4 mRNA expression increased 11- to 42-fold, while CYP2C9 and CYP2B6 mRNA expression increased by 1.7- to 2.7-fold and 2.5- to 2.9-fold, respectively (Figure 5, top). Corresponding increases in enzyme activity were 4- to 16-fold for CYP3A4, 1.7- to 3.2-fold for CYP2C9, and 3.0- to 5.0-fold for CYB2B6. Surprisingly, CYP1A2 enzyme activity also increased (2.6- to 6.3-fold) (Figure 5, bottom). Luciferase assays revealed that dicloxacillin activated PXR, but not CAR or AhR, in a dose-dependent manner (Figure 6). Similar trends were seen for the chemically related isoazolylpenicillin flucloxacillin (Figure 1), with increases in expression and activity of CYP3A4, CYP2C9, CYP2B6 and CYP1A2 (Supplementary Figures 5-8). The induction of P450 enzymes by flucloxacillin was less pronounced than dicloxacillin, which may be caused by a lower extent of PXR activation by flucloxacillin (Figure 6).
Discussion

This study confirms the inductive potential of the isoxazolyl penicillin dicloxacillin on expression and activity of drug-metabolising P450 enzymes. CYP3A4-, CYP2C9- and CYP2C19-mediated metabolism were induced by 10 days of treatment with 1g dicloxacillin three times daily in a five-drug clinical pharmacokinetic study in healthy men. From experiments in human hepatocytes, we show that the mechanism for this induction is likely PXR activation leading to increased expression and activity of P450 enzymes and we hypothesize that this extends to flucloxacillin.

The primary weakness of the study is the use of midazolam as a buccal administration. This may lead to larger variability in midazolam pharmacokinetics than intravenous administration but the randomization procedure serves to minimize the impact of within subject variability of midazolam absorption profile. While absolute bioavailability following buccal administration in adults is about 75%, it is unknown how much is absorbed by intestinal absorption [31]. As CYP3A4 is highly expressed in both the liver and the intestines, the increased midazolam metabolism observed in this study is likely caused by a combination of induction of hepatic and intestinal first-pass metabolism. In our study, we are unable to discriminate between intestinal and hepatic CYP3A4 induction. As we likely capture less effect on intestinal CYP3A4 activity, our results may reflect some conservative quantitative bias. While not statistically significant, the AUC ratio of midazolam to metabolite did suggest a change, but the precision of the estimate was wide. AUC estimation of metabolite is subject to several sources of bias, specifically with respect to the proportion, variation and rate of renal elimination of the glucuronidated metabolite, all of which affects metabolite AUC. Quantification of the amount of metabolite in the urine following deglucuronidation is therefore more robust and provides a better estimate of formation clearance and enzyme
activity. A minor weakness is the lack of protein analysis from the in vitro study. However, as both mRNA expression and activity of CYP enzymes were increased following dicloxacillin exposure, it is likely that protein levels also increased.

The main strength of this study is the full pharmacokinetic profiles in plasma and urine of five probe drugs and their main metabolites. This allows pharmacokinetic phenotyping of several P450 enzymes simultaneously and this extensive data provides an excellent overview of the different metabolic pathways induced by dicloxacillin. The clinical data from this study are supported by in vitro assessment in human hepatocytes, which is considered the gold standard for in vitro assessment of alterations in drug metabolism. Collectively, the in vivo and in vitro data strongly support dicloxacillin being a clinically relevant inducer of CYP3A4 and CYP2C metabolism.

The current results provide a mechanistic explanation for our previous observation that dicloxacillin decreased INR levels in patients receiving warfarin [14]. Dicloxacillin activates PXR, causing increased expression of CYP2C9 leading to increased CYP2C9-mediated metabolism of warfarin and lower clinical efficacy. CYP3A4 induction may be of significance as well, as the metabolism of warfarin R-enantiomer, a less potent inhibitor of vitamin-K epoxide reductase [32], is catalysed by CYP3A4 [33]. These results are also in line with previous in vitro data indicating dicloxacillin as a CYP3A4 inducer [17]. We show similar extent of induction of probe substrate metabolism for CYP3A4 and additionally CYP2C9.

Both omeprazole AUC$_{0-24h}$ and C$_{max}$ was significantly reduced after dicloxacillin exposure, though CL$_f$ for the 5-OH metabolite was unchanged. This was likely caused by one outlier with a large reduction in formation clearance (3-fold) after dicloxacillin exposure (Figure 4).
This is in stark contrast to increased CL\textsubscript{f} observed for the majority of other subjects (two of the other 11 individuals had minor reductions in CL\textsubscript{f} after dicloxacillin exposure, Figure 4). Interestingly, we show that dicloxacillin also decreased the AUC of dextromethorphan.

Besides CYP2D6, CYP3A4 also catalyzes the metabolism of dextromethorphan [34]. As CYP2D6 is not thought to be significantly inducible and CL\textsubscript{f} of dextrorphan is unchanged, the observed effect on dextrormethorphan pharmacokinetics is likely caused by induction of 3-methoxymorphinan formation by CYP3A4. Caffeine AUC is also affected, but no change in CL\textsubscript{f} was observed. CYP1A2 is responsible for around 95% of the primary metabolism of caffeine, but CYP3A4, CYP2C8 and CYP2C9 may also be involved in the metabolism [35]. It is plausible that induction of these enzymes may lead to the marginally increased caffeine metabolism observed in this study. This study did not assess the length of the observed induction after conclusion of dicloxacillin treatment. A previous study showed that after seven days of treatment with rifampicin, induction was no longer detectable eight days after conclusion of rifampicin treatment [36]. Another clinical study showed that the inductive effect of rifampicin on CYP3A4 persisted for about 4 weeks following 28 days treatment [37].

Due to the brief nature of exposure to dicloxacillin (standard treatment duration is 7 to 10 days), P450 induction is not expected to be of substantial clinical significance for a large proportion of widely used drugs. The clinical efficacy of many drugs such as cholesterol-lowering agents is largely a result of long-term treatment, and 10 days of induction should not have a substantial impact on the overall therapeutic efficacy. However, the clinical efficacy of drugs with a narrow therapeutic range, such as antiepileptics or immunosuppressants, may be affected by concomitant dicloxacillin treatment. Penicillins were previously suspected of causing therapeutic failure of oral contraceptives [38], but a potential mechanism for this
observation was never identified nor has any solid evidence substantiated the existence of these putative drug-drug interactions. Due to the substantial induction of CYP3A4 by dicloxacillin, it is plausible that concomitant use of dicloxacillin and oral contraceptives increases metabolism of oral contraceptives and may lead to risk of therapeutic failure and unwanted pregnancy. This putative drug-drug interaction warrants properly designed pharmacokinetic studies to determine the magnitude and clinical relevance.

Our in vitro results lend to a hypothesis that treatment with the chemically related drug flucloxacillin (Figure 1, more widely used in the UK [39,40]) may induce P450 enzymes, though possibly to a lesser extent than dicloxacillin. Induction of P450s by flucloxacillin should be quantified by an adequately designed in vivo pharmacokinetic drug-drug interaction study to guide recommendations for the use of these isoxazolyl penicillins. We also show that dicloxacillin induces CYP2B6 expression and activity. Whether this translates to changes in the clinical pharmacokinetics of CYP2B6 substrates needs to be elucidated, and is especially relevant for antiretroviral drugs such as efavirenz.

In conclusion, dicloxacillin is an inducer of CYP3A4- and CYP2C-mediated metabolism in healthy volunteers and in human hepatocytes. The effect is clinically relevant, and we recommend caution when prescribing dicloxacillin to users of drugs with a narrow therapeutic window that are metabolised by CYP2C9, CYP2C19 or CYP3A4.
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Conflicts of interest

The authors have no conflicts of interests.
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Hyperlinks used for Stage et al. 2017

P-glycoprotein, ABCB1, MDR1
http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=152#768

Pregnane X receptor, NR112
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=606

CYP3A4
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1337

CYP2C9
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1326

CYP2C19
http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=262#1328

CYP1A2
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1319

CYP2D6
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1329&familyId=262&familyType=ENZYME

Constitutive androstane receptor
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=607

 Aryl hydrocarbon receptor
http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2951
Table 1. Non-compartmental pharmacokinetic analysis of probes shows increased CYP3A4-, CYP2C9- and CYP2C19-mediated metabolism.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>Without dicloxacillin</th>
<th>With dicloxacillin</th>
<th>GMR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolbutamide (CYP2C9)</td>
<td>AUC₀.₂₄ₕ (ng*h/ml)</td>
<td>621,507 (505,775-744,162)</td>
<td>404,391 (355,015-582,480)</td>
<td>0.73 (0.65-0.81)</td>
</tr>
<tr>
<td></td>
<td>Cₘₐₓ (ng/ml)</td>
<td>51.706 (48,907-54,467)</td>
<td>49.130 (47,739-52,615)</td>
<td>0.93 (0.87-1.00)</td>
</tr>
<tr>
<td></td>
<td>T₁/₂ (hours)</td>
<td>9.0 (6.8-13.5)</td>
<td>6.1 (4.8-9.2)</td>
<td>0.72 (0.65-0.79)</td>
</tr>
<tr>
<td></td>
<td>CLₑ (L/h)</td>
<td>0.06 (0.05-0.12)</td>
<td>0.12 (0.09-0.16)</td>
<td>1.64 (1.44-1.87)</td>
</tr>
<tr>
<td>Omeprazole (CYP2C19)</td>
<td>AUC₀.₂₄ₕ (ng*h/ml)</td>
<td>228.4 (188.8-343.7)</td>
<td>80.2 (63.7-101.1)</td>
<td>0.33 (0.24-0.45)</td>
</tr>
<tr>
<td></td>
<td>Cₘₐₓ (ng/ml)</td>
<td>149.5 (105.5-242.2)</td>
<td>58.0 (42.8-77.3)</td>
<td>0.40 (0.25-0.66)</td>
</tr>
<tr>
<td></td>
<td>T₁/₂ (hours)</td>
<td>1.5 (1.2-3.5)</td>
<td>3.5 (2.2-5.6)</td>
<td>1.62 (0.71-3.71)</td>
</tr>
<tr>
<td></td>
<td>CLₑ (L/h)</td>
<td>1.9 (0.5-4.3)</td>
<td>3.7 (1.7-7.5)</td>
<td>2.01 (0.69-5.89)</td>
</tr>
<tr>
<td>Midazolam (CYP3A4)</td>
<td>AUC₀.₂₄ₕ (ng*h/ml)</td>
<td>37.4 (31.9-44.0)</td>
<td>23.5 (14.6-29.1)</td>
<td>0.54 (0.41-0.72)</td>
</tr>
<tr>
<td></td>
<td>Cₘₐₓ (ng/ml)</td>
<td>12.0 (9.6-13.7)</td>
<td>8.9 (6.2-12.5)</td>
<td>0.77 (0.59-1.01)</td>
</tr>
<tr>
<td></td>
<td>T₁/₂ (hours)</td>
<td>7.6 (5.5-9.9)</td>
<td>7.7 (6.3-8.5)</td>
<td>1.08 (0.87-1.33)</td>
</tr>
<tr>
<td></td>
<td>CLₑ (L/h)</td>
<td>38 (31-43)</td>
<td>67 (42-80)</td>
<td>1.59 (1.23-2.06)</td>
</tr>
<tr>
<td>Dextromethorphan (CYP2D6)</td>
<td>AUC₀.₂₄ₕ (ng*h/ml)</td>
<td>12.0 (5.4-24.5)</td>
<td>4.5 (2.7-11.2)</td>
<td>0.52 (0.32-0.83)</td>
</tr>
<tr>
<td></td>
<td>Cₘₐₓ (ng/ml)</td>
<td>1.15 (0.74-2.5)</td>
<td>0.76 (0.65-0.95)</td>
<td>0.69 (0.54-0.89)</td>
</tr>
<tr>
<td></td>
<td>T₁/₂ (hours, n=6)</td>
<td>13.9 (12.4-18.2)</td>
<td>21.4 (11.8-25.1)</td>
<td>1.25 (0.53-2.93)</td>
</tr>
<tr>
<td></td>
<td>CLₑ (L/h)</td>
<td>771 (275-1,927)</td>
<td>1,561 (483-2,800)</td>
<td>1.57 (0.97-2.55)</td>
</tr>
<tr>
<td>Caffeine* (CYP1A2)</td>
<td>AUC₀.₂₄ₕ (ng*h/ml)</td>
<td>22447 (20671-27837)</td>
<td>19358 (14563-23851)</td>
<td>0.81 (0.71-0.92)</td>
</tr>
<tr>
<td></td>
<td>Cₘₐₓ (ng/ml)</td>
<td>3,185 (2,936-3,890)</td>
<td>3,213 (2,838-3,422)</td>
<td>0.92 (0.81-1.04)</td>
</tr>
<tr>
<td></td>
<td>T₁/₂ (hours)</td>
<td>4.5 (3.9-4.6)</td>
<td>4.0 (3.0-4.8)</td>
<td>0.88 (0.79-0.99)</td>
</tr>
<tr>
<td></td>
<td>CLₑ (L/h)</td>
<td>0.45 (0.33-0.50)</td>
<td>0.47 (0.38-0.62)</td>
<td>1.17 (0.98-1.39)</td>
</tr>
</tbody>
</table>

Data are shown as medians with interquartile ranges. *Only showing pharmacokinetic data for 10 individuals for
caffeine. AUC: Area under the plasma concentration-time curve; $C_{\text{max}}$: Maximum plasma concentration; $\text{CL}_f$: Formation clearance of the main metabolite.
Figure 1. The chemical structure of isoxazolyl penicillins.
Figure 2. Flowchart of clinical study with 12 healthy men. All individuals completed the study.
Figure 3. Plasma concentration-time curves for tolbutamide, midazolam, omeprazole, caffeine and dextromethorphan with and without dicloxacillin. Dicloxacillin caused statistically significant reduction in all area under the plasma concentration-time curves.

Under figure: The solid coloured line is mean plasma concentration before dicloxacillin and the dashed black line is mean plasma concentration after dicloxacillin exposure. Only 10 individuals were included in the caffeine group due to violation of protocol.
Figure 4. Increased formation clearances were observed for tolbutamide and midazolam indicating increased CYP2C9- and CYP3A4-mediated metabolism.
Under figure: CL₄: Formation clearance. CL₄ was calculated as: Amount of metabolite in urine₀₋₄ / AUC of substrate₀₋₄.

One CYP2D6 poor metaboliser was excluded from the dextromethorphan graph to avoid clustering of the remaining individuals. Two individuals were excluded from the caffeine analysis due to ingestion of caffeine during the study. Dashed lines represent CYP2C9 poor metabolisers for tolbutamide and CYP2C19 ultrarapid metabolisers for omeprazole.
**Figure 5.** Increased gene expression (top panel) and activity (bottom panel) of CYP3A4, CYP2C9 and CYP2B6 caused by dicloxacillin treatment of human hepatocytes (mean of four donors). CYP1A2 expression was unchanged, but enzyme activity increased.
Under figure: Data are shown as mean fold increase relative to vehicle (0.1% DMSO) with standard deviations. Solid and dashed lines represent $C_{avg}$, $C_{ss,min}$ and $C_{ss,max}$ at 500 mg dicloxacillin 4 times daily in healthy volunteers [4].
Figure 6. Dicloxacillin activates pregnane X receptor, leading to the observed increase in expression and activity of CYP2C9, CYP2C19 and CYP3A4.

Under figure: PXR: Pregnane X receptor, AhR: Aryl hydrocarbon receptor, CAR: Constitutive androstane receptor. Data are shown as mean fold-increase relative to vehicle (0.1% DMSO) with standard deviations.

Solid and dashed lines for dicloxacillin represent $C_{\text{avg}}$, $C_{\text{ss,min}}$ and $C_{\text{ss,max}}$ at 500 mg dicloxacillin 4 times daily in healthy volunteers $^3$ while the solid line for flucloxacillin represents $C_{\text{max}}$ at 750 mg single dose of flucloxacillin in healthy volunteers $^3$. 