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

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AIM
The aim of this study was to study potential cytochrome P450 (CYP) induction by dicloxacillin.

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

RESULTS
A total of 10 days of treatment with dicloxacillin resulted in a clinically and statistically significant reduction in the area under the plasma concentration–time curve from 0 to 24 h for 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 the induction of CYP2C- and CYP3A4-mediated metabolism by dicloxacillin. Investigations in primary hepatocytes showed a statistically significant dose-dependent increase in CYP expression and activity by dicloxacillin, caused by activation of the 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.
**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* [1]. As dicloxacillin exhibits strong time-dependent antibacterial activity, the time above the minimal inhibitory concentration (0.125 μg ml⁻¹) is crucial for its clinical effect [2]. Due to the short elimination half-life of dicloxacillin (60–90 min after oral ingestion [3, 4]), administration every 6–8 h is necessary to sustain sufficient bactericidal concentrations. Following an oral dose of 500 mg dicloxacillin administered four times daily, plasma concentrations fluctuate from 2 μM, the minimum plasma concentration (Cmin), to 57 μM, the maximum plasma concentration (Cmax) [4]. Data on the utilization patterns of beta-lactamase-resistant penicillins in general, and dicloxacillin specifically, are scarce [5]. Dicloxacillin is the dominant choice of isoxazolyl penicillin 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], although it is unclear which cytochrome P450 (CYP) 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 the 5-hydroxy metabolite. This is likely to be caused by a combination of induction of CYP 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 a decreased international normalized 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 treatment resulted in markedly decreased INR values during warfarin therapy [14], leading to subtherapeutic INR levels in 60% of the treated patients. Additional case reports have described that initiation of treatment with another isoxazolyl penicillin, fluocloxacinil, 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 CYP enzymes and subsequent increased metabolism may provide an explanation for the above-mentioned drug–drug interactions, but this has not been confirmed in clinical pharmacokinetic studies.

The objective of the present 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 the expression and activity of CYP enzymes and nuclear receptor activation by dicloxacillin.

**Methods**

**Clinical study**

The study was designed as an open-label, randomized, two-phase, five-drug clinical pharmacokinetic cocktail study to assess the potential of dicloxacillin to induce CYP enzymes. We used a validated and previously used five-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 CYP-specific probes. We used a modified Cooperstown cocktail (midazolam, omeprazole, dextromethorphan and caffeine) [22]. As (S)-warfarin (the most pharmacologically active isomer of racemic warfarin) is metabolized by CYP2C9, we added tolbutamide, a well-validated substrate and...
biomarker for CYP2C9 metabolism [23], to the cocktail. This combination of biomarker drugs has previously been validated [19] and used to evaluate the changes in drug metabolism caused by drug–drug interactions [20, 21].

**Study medication and dose.** Tolbutamide (Arcosol® one 500 mg tablet, Meda AS, Allerød, Denmark), dextromethorphan (Dexofan® one 30 mg tablet, Takeda Pharma, Taastrup, Denmark), omeprazole (Omeprazol® 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 min 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 1 h before or at least 2 h after a meal. This is the dicloxacillin dosage regimen for the 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 five-drug cocktail in both phases after 12 h of fasting. Women were excluded owing 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 five-drug cocktail was ingested as in phase A. There was a washout period of at least 6 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 h. Urine was collected at 0–12 h and 12–24 h for determination of metabolites. The participants were given a meal 3 h after ingesting the drugs. The inclusion criteria for the study were: nonsmoking healthy men aged 18–55 years, body mass index (BMI) 18.5–29.9 kg m⁻² and estimated glomerular filtration rate (eGFR), alanine transaminase (ALT), bilirubin, haemoglobin and glycosylated haemoglobin A1c (HbA1c) within the respective reference ranges. Exclusion criteria for the study were: known allergies to any of the used drugs; known penicillin allergy or type 1 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.

Health 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 randomization using Sealed Envelope, which is freely available at www.sealedenvelope.com. The list was then made accessible to trial investigators using REDCap [24].

**Study approval.** 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 2016-043-478), 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 CYP isoenzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) was performed in plasma and urine samples. The samples were analysed for caffeine, paraxanthine, omeprazole, 5-hydroxyomeprazole, tolbutamide, 4-hydroxytolbutamide, dextromethorphan, dextorphan, midazolam and α-hydroxymidazolam. The analysis of plasma was performed according to the method described by Wohlforth 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 the addition of the isotope-labelled internal standard and dilution with the mobile phase before injection into the LC–MS/MS system. Analysis of omeprazole and its metabolite in urine was performed prior to the deglucuronidation procedure as

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**Figure 2**
Flowchart of clinical study with 12 healthy men. All individuals completed the study

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Statistics and pharmacokinetic analysis. The primary endpoint of the study was the accuracy in the area under the plasma concentration–time curve from 0 to 24 h (AUC wynh) for tolbutamide with and without dicloxacillin. To detect a difference of 25% in tolbutamide AUC wynh 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. Demographic data are shown as median values 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-tests and accepted at P < 0.05. Pharmacokinetic data were analysed by noncompartmental analysis using the R package NCAPPC [25]. The AUC wynh was estimated using the linear-up/logarithmic-down method. The formation clearance (CLf) and renal clearance (CLR) were estimated using the following equations:

\[
CL_f = \frac{\text{Amount of metabolite in urine}}{\text{Amount of substrate in urine}}
\]

\[
CL_R = \frac{\text{Amount of substrate in urine}}{\text{Amount of metabolite in urine}}
\]

Due to the short elimination half-lives of omeprazole and midazolam, 0–12 h CLf and CLR 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 have been described in detail previously [26]. Briefly, cryopreserved human hepatocytes [male lots: HU1765 (ThermoFisher catalogue #HMCPI, ThermoFisher Scientific, Waltham, MA, USA), HUM4034 (Triangle Research Laboratories catalogue #HUCPI, ThermoFisher Scientific, Waltham, MA, USA)]; female lots: HH1057 (In Vitro ADMET Laboratories (Columbia, MD, USA) catalogue #B2006), OII (Bioreclamation IVT (Westbury, NY, USA) catalogue #F0099S-P) were plated at 70 000 cells/well in collagen-coated 96-well plates in CHRM™ medium (ThermoFisher Scientific, Waltham, MA, USA) to obtain a monolayer of cells. After 4 h of incubation, the cells were overlaid with cold incubation media containing 0.35 mg ml⁻¹ GelTrex® (ThermoFisher Scientific, Waltham, MA, USA). The following day, the medium was replaced with fresh medium and incubated for another 24 h. On day 3, the cells were incubated for 48 h with dicloxacillin, fluroxacinil, rifampicin (PXR agonist, CYP3A and CYP regulation), phenobarbital [constitutive androstane receptor (CAR) agonist, CYP2B6 regulation] or 3-methylcholanthrene [aryl hydrocarbon receptor (AhR) agonist, CYP1A1 regulation] in increasing concentrations or with vehicle [0.1% dimethyl sulfoxide (DMSO)].

Nomenclature of targets and ligands Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [30], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [31–33].
Results

Clinical study
The study was an open, two-phase, randomized, clinical pharmacokinetic crossover study in 12 healthy men (Figure 2). The median age was 22 years (IQR 21–24; range 21–29 years) and the median BMI was 24.3 kg m⁻² (IQR 23.3–25.6; range 20.4–28.7). A five-drug cocktail of midazolam (CYP3A4) (2.5 mg buccal), tolbutamide (CYP2C9) (500 mg tablet), omeprazole (CYP2C19) (20 mg enteric capsule), caffeine (CYP1A2) (two 100 mg tablets) and dextromethorphan (CYP2D6) (30 mg tablet) was administered before and after 10 days of treatment with 1 g dicloxacillin three times daily. The sequence of study phases was randomized 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, all of which are well-described adverse reactions [34]. 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 Table S1. One CYP2D6 poor metabolizer, two CYP2C19 ultrarapid metabolizers and two CYP2C9 poor metabolizers were included in the study. The main analysis presented here is intent-to-treat, including all subjects, regardless of genotype. A sensitivity analysis which excluded individuals carrying altered function variants did not affect the conclusions of the study (Table S2).

Pharmacokinetics
AUC₀–₂₄ h, Cmax, elimination half-life (T½) and CLf 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 values of CLf with and without dicloxacillin exposure are shown in Figure 4. Detailed pharmacokinetic results are shown in Table S3, 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 as they violated the protocol by ingesting caffeine during the trial.

With concomitant dicloxacillin treatment, the AUC₀–₂₄ h of all probe drugs was significantly reduced, without changes in CLR (Table 1, Table S3, Figure 3). Tolbutamide AUC₀–₂₄ h and Cmax were reduced by 27% and 7%, respectively, driven by increased formation of the 4-hydroxytolbutamide metabolite, as reflected in a 64% increase in the CLf of the metabolite.

Table 1
Noncompartmental pharmacokinetic analysis of probes

<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></td>
<td>AUC₀–₂₄ h (ng*h ml⁻¹)</td>
<td>621 507 (505775–744 162)</td>
<td>404 391 (355015–582 480)</td>
<td>0.73 (0.65, 0.81)</td>
</tr>
<tr>
<td>Tolbutamide (CYP2C9)</td>
<td>Cmax (ng ml⁻¹)</td>
<td>51 706 (48907–54 467)</td>
<td>49 130 (47739–52 615)</td>
<td>0.93 (0.87, 1.00)</td>
</tr>
<tr>
<td></td>
<td>Tₚ (h)</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>CLf (l h⁻¹)</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>Cmax (ng ml⁻¹)</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>Tₚ (h)</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>CLf (l h⁻¹)</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>Cmax (ng ml⁻¹)</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>Tₚ (h)</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>CLf (l h⁻¹)</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>CLf (l h⁻¹)</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>Cmax (ng ml⁻¹)</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>Tₚ (h)</td>
<td>11.5 (7.4–2.5)</td>
<td>0.76 (0.65–0.95)</td>
<td>0.69 (0.54, 0.89)</td>
</tr>
<tr>
<td></td>
<td>CLf (l h⁻¹)</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>CLf (l h⁻¹)</td>
<td>771 (275–1927)</td>
<td>1561 (483–2800)</td>
<td>1.57 (0.97, 2.55)</td>
</tr>
<tr>
<td>Caffeine* (CYP1A2)</td>
<td>Cmax (ng ml⁻¹)</td>
<td>22 447 (20671–27 837)</td>
<td>19 358 (14563–23 851)</td>
<td>0.81 (0.71, 0.92)</td>
</tr>
<tr>
<td></td>
<td>Tₚ (h)</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>CLf (l h⁻¹)</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. AUC₀–₂₄ h, area under the plasma concentration–time curve from 0 to 24 h; CI, confidence interval; Cmax, maximum plasma concentration; CLf, formation clearance of the main metabolite; CYP, cytochrome P450; GMR, geometric mean ratio; Tₚ, half-life

*Pharmacokinetic data for only 10 individuals for caffeine are shown.
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Figure 3
Plasma concentration–time curves for tolbutamide (A), omeprazole (B), midazolam (C), caffeine (D) and dextromethorphan (E) with and without dicloxacillin. Only 10 individuals were included in the caffeine group owing to a violation of the protocol.

(Figure 4). Omeprazole AUC$_{0-24\text{ h}}$ and C$_{\text{max}}$ were 67% and 60% lower, respectively, with dicloxacillin exposure. Although the CL$_{\text{f}}$ of the CYP2C19-mediated 5-hydroxyomeprazole metabolite was twice as high with dicloxacillin exposure, this was not statistically significant ($P = 0.18$; geometric mean ratio [GMR] [95% confidence interval (CI): 2.01 (0.69, 5.89)]. Dicloxacillin treatment also led to a 46% reduction in midazolam AUC$_{0-24\text{ h}}$, attributed to increased CYP3A4 metabolism, as reflected by a 59% increase in the CL$_{\text{f}}$ of the -hydroxymidazolam metabolite. The ratio of the midazolam-to-metabolite AUC$_{0-24\text{ h}}$ increased slightly, with significant imprecision [GMR (95% CI): 1.27 (0.89, 1.80)]. Dicloxacillin

Figure 4
Formation clearance (CL$_{\text{f}}$) for tolbutamide and midazolam with and without dicloxacillin. CL$_{\text{f}}$ was calculated as: Amount of metabolite in urine$_{0-\text{t}}$/AUC of substrate$_{0-\text{t}}$, where AUC is the area under the plasma concentration–time curve and t is time. One cytochrome P450 m(CYP) 2D6 poor metabolizer was excluded from the dextromethorphan graph, to avoid clustering of the remaining individuals. Two individuals were excluded from the caffeine analysis owing to ingestion of caffeine during the study. Dashed lines represent CYP2C9 poor metabolizers for tolbutamide and CYP2C19 ultrarapid metabolizers for omeprazole.
also caused minor changes in caffeine pharmacokinetics; caffeine AUC$_{0\text{-}24}$ h was 19% lower with dicloxacillin with no change in Cl$_f$, suggesting that the changes in AUC$_{0\text{-}24}$ h were not caused by increased CYP1A2-mediated paraxanthine metabolism. Dicloxacillin also caused a marked 48% decrease in dextromethorphan AUC$_{0\text{-}24}$ h.

**Human hepatocytes**

The effect of dicloxacillin on the expression and activity of CYP3A4, CYP2C9, CYP2B6 and CYP1A2 was assessed in cryopreserved hepatocytes from four donors (two male and two female). Data from all donors are shown in Figure 5, and individual data from the four donors are shown in Figures S1–S4. All data are shown relative to the 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 5A). CYP3A4 mRNA expression increased 11.0- to 42.0-fold, while CYP2C9 and CYP2B6 mRNA expression increased by 1.7- to 2.7-fold and 2.5- to 2.9-fold, respectively (Figure 5A). Corresponding increases in enzyme activity were 4.0- to 16.0-fold for CYP3A4, 1.7- to 3.2-fold for CYP2C9, and 3.0- to 5.0-fold for CYP2B6. Surprisingly, CYP1A2 enzyme activity also increased (2.6- to 6.3-fold) (Figure 5A). 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 isoxazolyl penicillin, flucloxacillin (Figure 1), with increases in the expression and activity of CYP3A4, CYP2C9, CYP2B6 and CYP1A2 (Figures S5–S8). The induction of CYP enzymes by flucloxacillin was less pronounced than with dicloxacillin, which may have been caused by a decrease in PXR activation by flucloxacillin (Figure 6).

**Discussion**

The present study confirmed the inductive potential of the isoxazolyl penicillin, dicloxacillin, on the expression and activity of drug-metabolizing CYP enzymes. CYP3A4-, CYP2C9- and CYP2C19-mediated metabolism were induced by 10 days of treatment with 1 g dicloxacillin three times daily in a five-
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Dicloxacillin induces CYP2C- and CYP3A-mediated drug 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 the warfarin R-enantiomer, a less potent inhibitor of vitamin K epoxide reductase [36], is catalysed by CYP3A4 [37]. These results are also in line with previous in vitro data indicating that dicloxacillin is a CYP3A4 inducer [17]. We showed a similar extent of induction of probe substrate metabolism for CYP3A4, and also CYP2C9.

Both omeprazole AUC$_{0-24\,\text{h}}$ and C$_{\text{max}}$ were significantly reduced after dicloxacillin exposure, although Cl$_{\text{f}}$ for the 5-hydroxy metabolite was unchanged. This was likely to have been caused by one outlier with a large reduction in Cl$_{\text{f}}$ (threelfold) after dicloxacillin exposure (Figure 4). This is in stark contrast to the increased Cl$_{\text{f}}$ observed for the majority of other subjects (two of the other 11 individuals had minor reductions in Cl$_{\text{f}}$ after dicloxacillin exposure; Figure 4). Interestingly, we showed that dicloxacillin also decreased the AUC$_{0-24\,\text{h}}$ of dextromethorphan. Besides CYP2D6, CYP3A4 also catalyses the metabolism of dextromethorphan [38]. As CYP2D6 is not thought to be significantly inducible and the Cl$_{\text{f}}$ of dextorphan is unchanged, the observed effect on dextromethorphan pharmacokinetics is likely to have been caused by induction of 3-methoxymorphinan formation by CYP3A4. Caffeine AUC$_{0-24\,\text{h}}$ is also affected but no change in Cl$_{\text{f}}$ 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 [39]. It is plausible that induction of these enzymes may lead to the marginally increased caffeine metabolism that we observed. The present study did not assess the length of the observed induction after conclusion of dicloxacillin treatment. A previous study showed that 7 days of treatment with rifampicin produced enzyme induction, but the induced activities were no longer detectable 8 days after conclusion of rifampicin treatment [40]. Another clinical study showed that the inductive effect of rifampicin on CYP3A4 persisted for about 4 weeks following 28 days of treatment [41].

Owing to the brevity of the exposure to dicloxacillin (the standard treatment duration is 7–10 days), CYP 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 antiepileptic agents or immunosuppressants, may be affected by concomitant dicloxacillin treatment. Penicillins were previously suspected of causing therapeutic failure of oral contraceptives [42] but a potential mechanism for this observation was never identified, and no solid evidence has substantiated the existence of these putative drug–drug interactions. Owing to the substantial induction of CYP3A4 by dicloxacillin, it is plausible that concomitant use of dicloxacillin and oral contraceptives increases the metabolism of the latter, and may lead to a risk of therapeutic failure and unwanted pregnancy. This putative drug–drug interaction warrants properly designed pharmacokinetic studies to determine its magnitude and clinical relevance.

Our in vitro results lend credence to a hypothesis that treatment with the chemically related drug, flucloxacillin (Figure 1), more widely used in the UK [43, 44], may induce CYP enzymes, although possibly to a lesser extent than dicloxacillin. Induction of CYPs 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 showed 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 metabolized by CYP2C9, CYP2C19 or CYP3A4.

Competing Interests

There are no competing interests to declare.

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Dicloxacillin induces CYP2C- and CYP3A-mediated drug metabolism


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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.


Figure S1 Effects of dicloxacillin on the expression and activity of cytochrome P450 (CYP) enzymes in cryopreserved hepatocytes from donor HU1765

Figure S2 Effects of dicloxacillin affects expression and activity of cytochrome P450 CYP enzymes in cryopreserved hepatocytes from donor TRL4034

Figure S3 Effects of dicloxacillin on the expression and activity of cytochrome P450 (CYP) enzymes in cryopreserved hepatocytes from donor OII

Figure S4 Effects of dicloxacillin on the expression and activity of cytochrome P450 (CYP) enzymes in cryopreserved hepatocytes from donor 1057

Figure S5 Effects of fluocxacillin on the expression and activity of cytochrome P450 (CYP) enzymes in cryopreserved hepatocytes from donor OII

Figure S6 Effects of fluocxacillin on the expression and activity of cytochrome P450 (CYP) enzymes in cryopreserved hepatocytes from donor TRL4034

Figure S7 Effects of fluocxacillin on the expression and activity of cytochrome P450 (CYP) enzymes in cryopreserved hepatocytes from donor 1057

Figure S8 Effects of fluocxacillin on the expression and activity of cytochrome P450 (CYP) enzymes in cryopreserved hepatocytes from donor OII

Table S1 Genotype analysis identified two cytochrome P450 (CYP) 2C9 ((CYP2C9*2/*3) and CYP2C9*3/*3) and one CYP2D6 (CYP2D6*4/*4) poor metabolizers and two CYP2C19 ultrarapid metabolizers (CYP2C19*17/*17)

Table S2 Sensitivity analysis of the noncompartmental analysis, excluding poor- and ultrarapid metabolizers of cytochrome P450 (CYP) 2C9, CYP2C19 and CYP2D6

Table S3 Results from noncompartmental analysis of all included individuals for all five probe drugs and their primary metabolites