

ARTICLE

Oral and intravenous pharmacokinetics of metformin with and without oral codeine intake in healthy subjects: A cross-over study

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Abstract

The aim of the study was to investigate if there is a clinically relevant drug interaction between metformin and codeine. Volunteers were randomized to receive on four separate occasions: (A) orally administered metformin (1 g), (B) intravenously administered metformin (0.5 g), (C) five doses of tablet codeine 25 mg; the last dose was administered together with oral metformin (1 g), and (D) five doses of tablet codeine 25 mg; the last dose was administered together with metformin (0.5 g) intravenously. Blood samples were drawn for 24 h after administration of metformin, and for 6 h after administration of codeine and analyzed using liquid chromatography and tandem mass spectrometry. Healthy volunteers genotyped as *CYP2D6* normal metabolizers (**1/*1*) without known reduced function variants in the *OCT1* gene (*rs12208357*, *rs34130495*, *rs34059508*, and *rs72552763*) were invited. The median absorption fraction of metformin was 0.31 and was not influenced by codeine intake. The median time to maximum concentration (T_{max}) after oral intake of metformin was 2 h without, and 3 h with codeine ($p = 0.06$). The geometric mean ratios of the areas under the plasma concentration time-curve (AUCs) for morphine and its metabolites M3G and M6G for oral intake of metformin-to-no metformin were 1.21, 1.31, and 1.27, respectively, and for i.v. metformin-to-no metformin 1.28, 1.34, and 1.30, respectively. Concomitant oral and i.v. metformin increased the plasma levels of morphine, M3G and M6G. These small pharmacokinetic changes may well contribute to an increased risk of early discontinuation of metformin. Hence, a clinically relevant drug-drug interaction between metformin and codeine seems plausible.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

We have previously reported in a large pharmacoepidemiologic study comprising 400,000 users of metformin that concomitant intake of codeine leads to early

discontinuation of metformin. Metformin is highly dependent on drug transporters to cross cell membranes. Organic cation transporter 1 (OCT1) transports metformin in (and possibly out) of the enterocytes and into the hepatocytes. Codeine and its main metabolite morphine are both inhibitors of the OCT1 transporter and the latter is also a substrate.

WHAT QUESTION DID THIS STUDY ADDRESS?

Is there a clinically relevant pharmacokinetic drug-drug interaction among metformin, codeine, and morphine?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Metformin increases morphine and metabolite levels.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The small pharmacokinetic changes observed in our study may well contribute to an increased risk of early discontinuation of metformin.

INTRODUCTION

Due to its efficacy, safety profile, and cardioprotective properties, metformin is the drug of choice¹ for pharmacological treatment of type 2 diabetes (T2D).

Much attention has been given to the importance of the gut for metformin's effect in humans² and multiple mechanisms have been proposed; one of which is increased utilization of glucose in the enterocytes² with increased lactate production as a result.^{3,4}

The uptake of metformin across the apical enterocyte membrane is facilitated by organic cation transporter 1 (OCT1), PMAT, and organic cation transporter 3 (OCT3).⁵ The hepatic uptake of metformin is facilitated by OCT1.⁶ Metformin is eliminated in the kidneys by both glomerular filtration and tubular secretion, the latter being facilitated by organic cation transporter 2 (OCT2) and the MATE1 and MATE2-K.⁷

In 2016, our group conducted a cohort study,⁸ which showed that concomitant intake of codeine led to early discontinuation (within 6 months after first prescription) of metformin. In accordance, other register-based studies have reported an increased risk of gastrointestinal intolerance in patients using metformin and codeine concomitantly.^{9,10} Codeine is a prodrug, and ~ 10% is O-demethylated by CYP2D6 to the active metabolite morphine, which is further metabolized to morphine-3-glucuronide (M3G) and the pharmacologically active morphine-6-glucuronide (M6G).^{11,12} Codeine and morphine are inhibitors of OCT1 and morphine is also a substrate.¹³ Hence, metformin, codeine, and morphine might affect each other's transport across cell membranes and therefore also each other's pharmacokinetics. This potential drug-drug interaction (DDI) may hypothetically contribute to early discontinuation of metformin.

The main purpose of this study was to investigate if codeine affects the absorption fraction of metformin. Thus, we developed an i.v. formulation of metformin and administered it to healthy volunteers. Our secondary purpose was to investigate if metformin affects the plasma levels of codeine and morphine.

A tertiary purpose was to compare the effect of oral and i.v. administration of metformin on glucose and lactate production with and without codeine.

METHODS

Study participants

Volunteers from our previous study¹⁴ were invited to participate provided they were genotyped as *CYP2D6* normal metabolizers (*1/*1) and did not have any of the single-nucleotide polymorphisms rs12208357, rs34130495, and rs34059508 or the deletion rs72552763 in *OCT1* known to cause reduced uptake of metformin.¹⁵ Details on the cohort can be found in Tables S1 and S2. All volunteers claimed to be healthy and did not ingest prescription-, herbal-, or over-the-counter medicine or supplements (birth control pills and regular vitamin supplements were accepted). All had a body mass index below 29.9 kg/m² and an age less than or equal to 30 years. None had a history of alcohol abuse or hypersensitivity to metformin, codeine, or morphine. Use of safe contraceptives was demanded for female volunteers and no pregnant or breastfeeding women were allowed to participate. Before administration of study medication, all women tested negative for pregnancy. We assessed the renal and liver function by measuring plasma creatinine, estimated glomerular filtration rate (eGFR) and alanine aminotransferase, respectively,

and all had to be within normal range or clinically insignificantly deviate from it. We also measured HbA1c, which was required to be within normal range. We obtained verbal and written informed consent from all volunteers included in the study.

Study design

The study was open label. Volunteers were scheduled to four study periods with minimum 1-week washout in between. The sequence was randomized for each volunteer.

A: A single oral dose of 1 g metformin hydrochloride, (780 mg of free base; Orion Pharma, Copenhagen, Denmark).

B: A single i.v. dose of 0.5 g metformin hydrochloride (390 mg of free base).

C: Five sequential oral doses of 25 mg codeine, phosphate sesquihydrate (17.6 mg of free base; Takeda Pharma, Taastrup, Denmark) plus a single oral dose of metformin (1 g), which was ingested together with the fifth dose of codeine.

D: Five sequential oral doses of 25 mg codeine, phosphate sesquihydrate (17.6 mg of free base plus a single i.v. dose of metformin (0.5 g) together with the fifth dose of codeine.

The randomization was carried out using REDCap (version 10.0.28; Vanderbilt University).¹⁶ In order to limit the number of trial sequences, the volunteers were block-randomized using a Latin square design to ABCD, BDAC, CABD, or DCBA.

Study periods

Volunteers fasted from midnight and were not allowed to use alcohol, medications (over-the-counter or prescription), vitamins, and herbal medication in the 24 h, or perform vigorous physical training in the 48 h leading up to the trial. Urine was collected from drug administration and the following 24 h in each trial period where metformin was ingested or injected (see above). A single peripheral venous catheter was inserted in vena mediana cubiti in order to draw blood samples. A second venous catheter was inserted in the opposite arm in study periods where volunteers were injected with metformin.

Blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after oral metformin ingestion. Metformin was injected over a 5-min period and additional samples were taken 5, 10, 20, and 40 min after the start of the injection and then every 20 min until 3 h.

Blood samples were drawn at 0, 1, 2, 3, 4, 5, and 6 h after the first codeine dose. The blood sample at 6 hours was drawn right before the ingestion of the second codeine tablet (14 p.m.). The third dose of codeine was ingested at 8 p.m. and, for practical reasons, the fourth dose was to be ingested before bedtime but no earlier than 11 p.m. The next morning, the fifth and the last dose of codeine was ingested together with either oral or i.v. metformin. Blood samples drawn at 0, 1, 2, 3, 4, 5, and 6 h after ingestion of the fifth dose of codeine with metformin were also measured for codeine and metabolites. Vacutainers with EDTA as anticoagulant were used for metformin and codeine blood sample collection while vacutainers with lithium heparin was used for glucose and lactate blood sample collection. Blood samples were immediately—to no longer than 45 min after blood sampling—centrifuged, and the plasma fraction was kept at -20°C until drug analysis. Lactate and glucose vacutainer tubes were kept on ice before and after blood sampling. Urine was collected and stored at -20°C until analysis.

Study medication

Intravenous metformin was specifically developed for this project by the hospital pharmacy at Odense University Hospital. Details on the preparation are provided in Text S1.

Food during the trial

Volunteers fasted at least 1 h after intake of codeine on the two trial days solely containing this drug. On the remaining trial days, volunteers were only allowed standardized meals prepared by the hospital kitchen at Odense University Hospital (Text S1). Fasting from midnight was adhered to on all trial days.

Study procedures

The study was approved by the Danish Medicines Agency (EudraCT no.: 2017-003857-40), OPEN at the University of Southern Denmark (no: OP_510) and approved by the Regional Committees on Health Research Ethics for Southern Denmark (J. no: S-20170166), and the Danish Data Protection Agency (J. no. 2012-58-0018). The trial was registered at www.clinicaltrials.gov (NCT03335423). The 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.

Analytical methods

The concentrations of metformin, codeine, morphine, M3G, M6G, and codeine-6-glucuronide (C6G) in plasma and urine samples were determined at the Department of Public Health, Clinical Pharmacology, Pharmacy and Environmental Medicine, University of Southern Denmark, by use of isotope dilution and liquid chromatography and tandem mass spectrometry. The metformin method has previously been described in detail.¹⁴ The lower limit of quantification (LOQ) for metformin in plasma and urine was 10 ng/ml. Within-day coefficient of variation and between-day reproducibility was less than 8%. A new analytical method was developed to measure codeine, morphine, M3G, M6G, and C6G in plasma. A detailed description is included in Text S2. Validation of the method was assessed at three concentrations (low, medium, and high) in quintuple determination over a period of 4 days. The calibration curves showed excellent and consistent linearity with a correlation coefficient r greater than 0.998 for all the compounds. The LOQ was 0.2 ng/ml for morphine, M3G, M6G, and codeine, and 2 ng/ml for C6G. The with-in batch precision (percent coefficient of variation [CV%]) was approximately less than 8% for morphine and M6G, approximately less than 6.5% for M3G and codeine, and less than 2% for C6G. The between batch precision (CV%) was approximately less than 9% for M6G, approximately less than 8% for codeine and C6G, approximately less than 4% for M3G, and approximately less than 11% for morphine. Plasma glucose and lactate were measured using an ABL800 FLEX Analyzer (Radiometer, Copenhagen, Denmark).

Statistical analysis and considerations

The descriptive data are presented as medians and range. The pharmacokinetic data are presented as medians with 25th and 75th percentile range (interquartile range [IQR]). Before statistical analysis, visually guided by Q_{norm} plots metformin volume of distribution (V), metformin's and codeine's half-life ($T_{1/2}$) and codeine's area under the plasma concentration time-curve (AUC) were logarithmically transformed to create a Gaussian distribution. A paired t -test was used to assess the effect on the pharmacokinetic parameters. Codeine's effect on metformin time to maximum concentration (T_{max}) and metformin's effect on AUC of morphine and its metabolites was determined using the nonparametric Wilcoxon signed-rank test.

The renal clearance (CL_{renal}) and total clearance (CL_{total}) results are not adjusted for the glomerular filtration rate (GFR_i) because the eGFR is only measured at baseline and the volunteers are their own control.

Geometric mean ratios (GMRs) were calculated by logarithmic transformation of AUCs, subtracting $AUC_{steady-state}$ from $AUC_{single-dose}$, calculating the mean from the product and taking the antilog of the end result.

Pharmacokinetic data analysis

All pharmacokinetic parameters were calculated by noncompartmental methods using the software package "NCAPPC" in R version 3.6.3 and Stata version 16.1 (StataCorp, College Station, TX, USA).

The AUC of metformin, lactate, glucose, and codeine plus its metabolites was calculated using the linear-up/logarithmic-down method. The percentage of AUC from zero to infinity ($AUC_{0-\infty}$) extrapolated was determined as C_{last}/ke . The actual blood sampling times were used for determination of all metformin and codeine parameters. The apparent half-life was calculated as follows:

$$T_{1/2} = \frac{\ln 2}{ke}, \quad (1)$$

where ke is the terminal slope of the log plasma concentrations versus the time plot calculated by linear regression with a minimum of three datapoints.

Metformin's CL_{total} was calculated as follows:

$$CL = \frac{\text{Dose}_{iv}}{AUC_{iv(0-\infty)}}. \quad (2)$$

Metformin's CL_{renal} after oral intake was calculated as follows:

$$CL_{renal} = \frac{\text{amount of metformin in urine}_{0-24h}}{AUC_{0-24h}}. \quad (3)$$

The absorption fraction of metformin (F) was calculated by the area method:

$$F = \frac{(AUC_{(0-\infty)oral}) * (\text{Dose}_{iv})}{(AUC_{0-\infty iv}) * (\text{Dose}_{oral})}. \quad (4)$$

Equation 4 is based on the assumption that clearance of metformin is the same after oral and i.v. administration. Metformin is exclusively eliminated by the kidneys, and after oral intake the absorption fraction (F_{oral}) can also be calculated by the renal excretion method:

$$F_{oral} = \frac{A_e}{\text{Dose}_{oral}}. \quad (5)$$

A_e is the amount of metformin excreted in the urine during 24 h after intake of an oral dose.

The volume of distribution, V , after i.v. administration was calculated according to:

$$V = CL/ke, \quad (6)$$

The oral clearance of codeine (CL/F) after a single dose of codeine, was calculated as follows:

$$CL/F = \frac{\text{Dose}}{AUC_{\text{codeine}}(0-\infty)}. \quad (7)$$

The CL/F in steady-state, was calculated as follows:

$$CL/F = \frac{\text{Dose}}{AUC_{\text{codeine}}(0-6h)}. \quad (8)$$

The oral volume of distribution (V/F) was calculated as follows:

$$\frac{T1/2 * CL/F}{\ln(2)}. \quad (9)$$

Sample size

Power was calculated pre hoc. With an expected mean AUC of metformin of 7091 ± 2050 h ng/ml,¹⁷ we needed 15 healthy subjects to detect a 30% difference in the AUC during codeine after oral intake with a power of 80% and an alpha of 5%.

RESULTS

Twenty-one volunteers were included. One volunteer was excluded halfway through the trial due to technical challenges with the blood sample collection. Four volunteers withdrew before the trial started. Sixteen completed the trial. Demographics are shown in Table 1.

The trial was self-controlled, and the volunteer who dropped out halfway through was excluded from all statistical analysis.

The pharmacokinetics and drug-drug interactions of metformin and codeine

The plasma concentrations of metformin versus time after i.v. and oral administration without and with codeine are shown in Figure 1 and the derived pharmacokinetic parameters are presented in Table 2. The median percentage of metformin $AUC_{0-\infty}$ extrapolated was 5% after oral

TABLE 1 Demographic information of 16 healthy volunteers

Demographic information	Median	Range (minimum: maximum)
Age at inclusion (years)	25	22–29
BMI (kg/m ²)	24	19–29
Plasma creatinine (μmol/L)	74	60–104
HbA1c (mmol/mol)	30	26–35
eGFR(mL/min/1.73m ²)	90	84–90
ALAT (U/L)	18	12–56
Gender: women 11, men 5		

Abbreviations: ALAT, alanine aminotransferase; BMI, body mass index weight/height²; eGFR, estimated glomerular filtration rate.

administration and 1% after i.v. administration. The median (25th–75th IQR) $T_{1/2}$ of metformin without codeine was 4.2 h (2.8–5.3 h) after oral administration, which is significantly longer than the median 2.3 h (2.1–2.8 h) observed after i.v. administration ($p = 0.001$), and the same applied when metformin was given with codeine (Table 2). After i.v. administration, the median total clearance of metformin was 30 and 31 L/h without and with codeine, respectively ($p = 0.9$). However, without and with codeine, the CL_{renal} after oral metformin intake was 42 L/h and 38 L/h, respectively (Table 2), which is significantly higher than the CL_{total} after i.v. administration ($p < 0.01$). Following i.v. administration, the amount of metformin excreted in urine was ~ 410 mg, which is 5% higher than the administered dose. This small systematic bias is most likely due to a combination of small deviances in the dose administered, measurement of urine weight, and in the dilution of urine in the analytical process. According to the US Food and Drug Administration's (FDAs) guideline,¹⁸ a drug has the potential to inhibit the OCT transporters in vivo if the $I_{\text{maxunbound}}/\text{half-maximal inhibitory concentration (IC50)}$ value is greater than or equal to 0.1, where $I_{\text{maxunbound}}$ is the maximum unbound plasma concentration of the inhibitor. Using our own maximum concentrations of orally administered codeine and metformin and an IC50 value for the OCT1 and OCT2 substrate MPP (as a value for metformin and morphine is lacking) our result in plasma was 0.01 for inhibition of MPP at the OCT1 transporter by codeine and 0.02 for inhibition of MPP at the OCT2 transporter by metformin. Codeine did have a theoretical potential of inhibiting OCT1 in the gut as the $I_{\text{maxunbound}}/\text{IC50}$ value was 32.

The median (25th--75th IQR) absorption fraction, F (Equation 4), was 0.31 (0.26–0.35) without codeine, and 0.34 (0.26–0.39) with codeine ($p = 0.9$). The median F_{oral} (Equation 5) was ~ 29% larger than the median of F (Equation 4) without codeine and ~ 6% larger with codeine ($p < 0.05$). We found no difference in the oral metformin

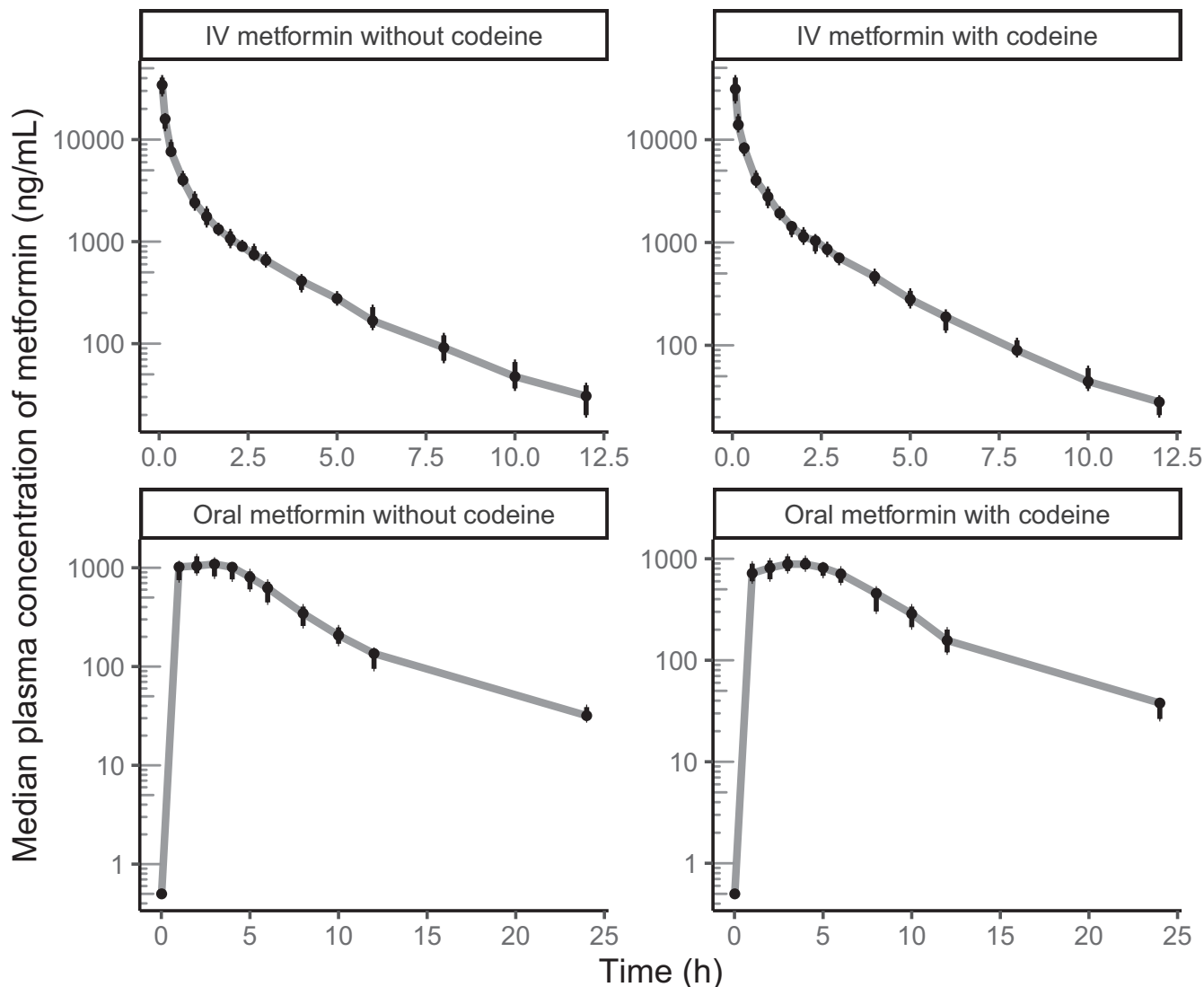


FIGURE 1 The median metformin plasma concentration vs time profile for i.v. and oral metformin in 16 healthy volunteers, with and without codeine. Data is presented with 25th–75th interquartile range (IQR) error bars

$AUC_{0-\infty}$ when metformin was ingested alone compared to concomitant intake of codeine (Figure 1 and Table 2). Nor did concomitant intake of codeine impact metformin's $T_{1/2}$, CL_{renal} , or maximum plasma concentration (C_{max}). Likewise, we found no difference in i.v. metformin $AUC_{0-\infty}$ or CL_{total} when administered alone compared to concomitant intake with codeine (Figure 1 and Table 2). However, the median (25th–75th IQR) time to reach the highest determined plasma concentration was 2 h (1.5–3 h) without codeine and 3 h with codeine (2–4 h, $p = 0.06$; Table 2). Codeine did not have an impact on the oral volume of distribution ($p = 0.6$; Table 2). After i.v. metformin, both its $T_{1/2}$ and the V of metformin was significantly smaller (4% and 15%, respectively) when taken together with codeine (Table 2). Two volunteers had a high V following i.v. metformin without codeine due to a low k_e . Removing these two outliers from the V analysis resulted in a median V

(25th–75th IQR) of 105 L (93–118) without codeine and 92 L (83–106) with codeine ($p = 0.2$). No statistical changes were observed for the $T_{1/2}$ when the two outliers were removed.

The plasma concentrations of codeine and metabolites are shown in Figure 2 and the pharmacokinetic parameters are shown in Table 3. Unexpectedly, codeine and its metabolites were detected in high concentrations in the first blood sample drawn ~ 9 h after the fourth and immediately before the fifth and last codeine dose (Figure 2). The median percentage of codeine $AUC_{0-\infty}$ extrapolated was 17%, whereas it was 25% for morphine and M6G, 24% for C6G, and 29% for M3G. Metformin intake did not affect the CL/F of codeine or AUC of codeine and C6G (Table 3). GMRs (95% confidence interval [CI]) of the AUCs for morphine and its metabolites M3G and M6G for oral intake of metformin-to-no metformin were 1.21 (1.00–1.46), 1.31

Administration and parameters	Without codeine	With codeine	<i>p</i> value
Pharmacokinetics after i.v. metformin			
$T_{1/2}$ (h)	2.3 (2.1–2.8)	2.2 (1.9–2.4)	0.02
C_{max} ($\frac{\text{ng}}{\text{ml}}$)	34,300 (27,900–40,900)	31,100 (23,400–40,600)	0.3
$AUC_{0-\infty}$ ($\frac{\text{ng} \cdot \text{ml}}{\text{h}}$)	12,800 (11,700–14,200)	12,700 (11,700–13,600)	0.7
V (L)	110 (94–120)	93 (84–106)	0.04
CL_{total} ($\frac{\text{L}}{\text{h}}$)	30 (27–33)	31 (28–33)	0.9
Pharmacokinetics after oral metformin			
CL_{renal} ($\frac{\text{L}}{\text{h}}$) ^a	42 (37–45)	38 (32–43)	0.5
$T_{1/2}$ (h)	4.2 (2.8–5.3)	3.7 (2.8–5.6)	0.8
C_{max} ($\frac{\text{ng}}{\text{ml}}$)	1180 (1020–1310)	996 (870–1190)	0.4
T_{max} (h)	2 (1.5–3)	3 (2–4)	0.06
$AUC_{0-\infty}$ ($\frac{\text{ng} \cdot \text{ml}}{\text{h}}$)	8300 (7060–9400)	8400 (6900–9500)	0.8
V/F	610 (340–710)	444 (358–897)	0.6
F	0.31 (0.26–0.35)	0.34 (0.26–0.39)	0.9
F_{oral} ^a	0.40 (0.34–0.48)	0.36(0.29–0.46)	0.9

Data is presented as medians with the 25th–75th interquartile range.

Abbreviations: $AUC_{0-\infty}$, area under the plasma concentration time-curve from zero to infinity; CL_{renal} , renal clearance; CL_{total} , total clearance; C_{max} , maximum plasma concentration of metformin; F , absorption fraction calculated by the area method; F_{oral} , absorption fraction calculated by the renal method; $T_{1/2}$, terminal half-life; V , volume of distribution; T_{max} , time to the maximal plasma concentration; V/F , oral volume of distribution.

^aStatistical test concerning CL_{renal} and F_{oral} are based on data from 13 volunteers. Two volunteers in the oral visit with codeine and one in the oral visit without codeine had incomplete urine collection. Following i.v. metformin administration without codeine, one volunteer had incomplete urine collection and one excreted far more metformin than was ingested, which is not pharmacologically possible and probably due to a technical error. One volunteer had incomplete urine collection following i.v. metformin administration with codeine. These volunteers were not included in the statistical analysis of CL_{renal} and F_{oral} .

(1.17–1.46), and 1.27 (1.15–1.39), respectively, and for i.v. metformin-to-no metformin were 1.28 (1.09–1.49), 1.34 (1.22–1.46), and 1.30 (1.18–1.40). However, the GMR of the AUC of morphine for oral intake of metformin-to-no metformin did not reach statistical significance ($p = 0.06$). The half-lives of codeine's metabolites were similar to the parent drug (Figure 2).

An overview of plasma lactate and glucose levels versus time profile after oral and i.v. metformin without codeine is presented in Figures 3 and 4. Thus, a peak increase in plasma lactate levels was observed at all visits after ~ 4 h. Codeine did not have an impact on lactate AUC from zero to 24 h (AUC_{0-24h}) neither with oral nor i.v. metformin ($p > 0.05$; data not shown). The lactate AUC_{0-24h} was ~ 2.3% larger ($p = 0.047$) after oral compared to i.v. administration of metformin without codeine. However, there was no statistical difference in lactate $AUC_{(0-3h),(0-12h)}$ ($p = 0.9$ and $p = 0.2$, respectively) after oral compared to i.v. administration of metformin without codeine. Accordingly, there was no effect on peak plasma

lactate levels at 4 h ($p = 0.9$). Plasma glucose AUC_{0-24h} was ~ 4% lower ($p = 0.03$) after oral compared to i.v. metformin administration without codeine, and the peak plasma glucose levels were significantly higher after i.v. compared to oral metformin ($p = 0.005$). No difference was observed when comparing glucose AUC for the first 3 hours after administration ($p = 0.2$).

DISCUSSION

This is the largest clinical pharmacokinetic study ever conducted of i.v. metformin administration in man. It is also the first pharmacokinetic DDI study between codeine and metformin. Previous pharmacokinetic studies on i.v. metformin^{19–21} found similar half-lives as we did. As expected, we found a longer plasma half-life after oral compared to i.v. administration, consistent with flip-flop kinetics (Table 2). Metformin has not been detected in feces after i.v. administration, which indicates that there

TABLE 2 The impact of concomitant intake of codeine and oral (1000 mg) or i.v. (500 mg) metformin on metformin's pharmacokinetic parameters in 16 healthy volunteers

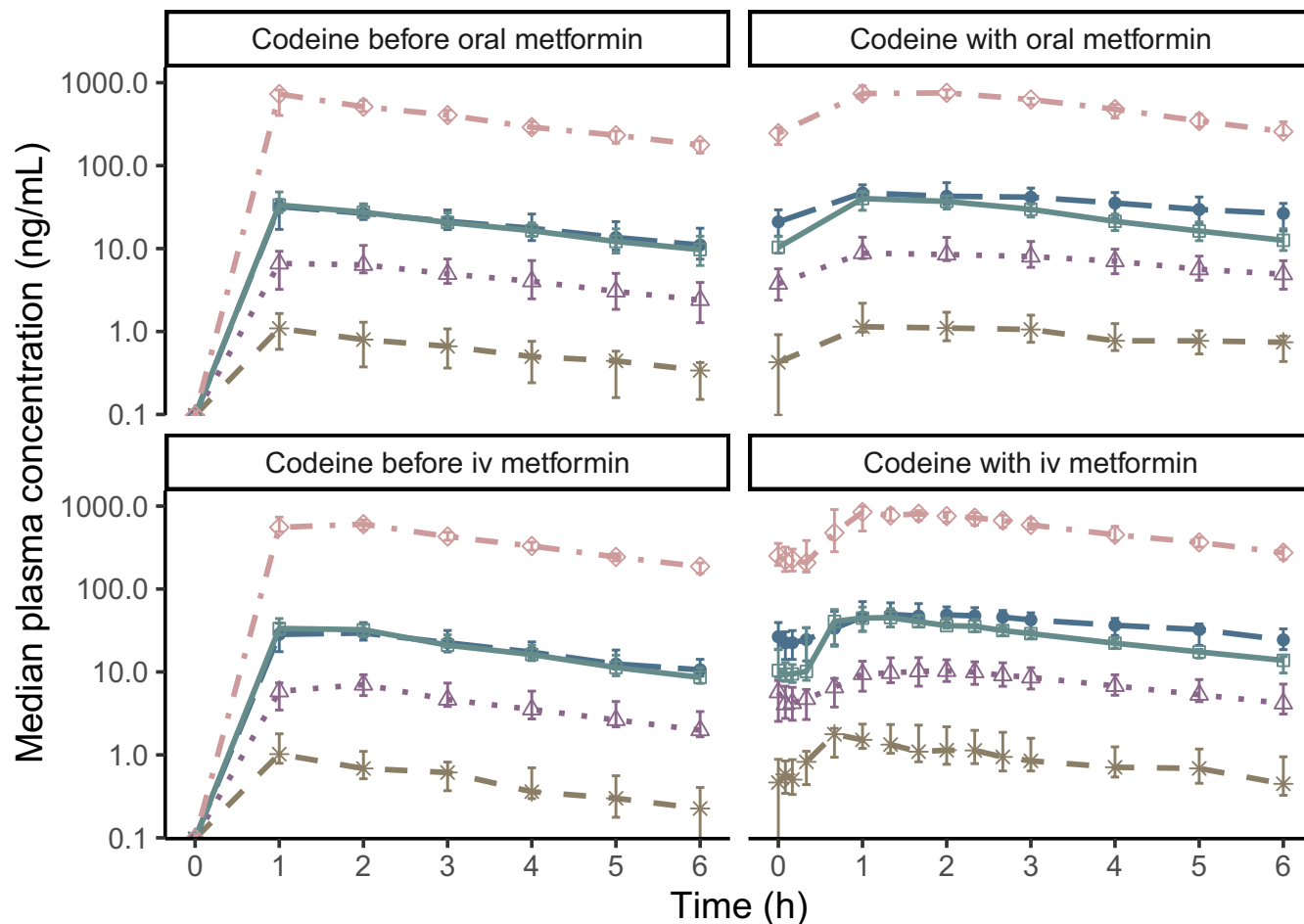


FIGURE 2 The median codeine and metabolite plasma concentration versus time profile for codeine before oral and i.v. metformin and codeine together with oral and i.v. metformin in 16 healthy volunteers. Data is presented with 25th–75th interquartile range (IQR) error bars. Brown with stars = morphine; blue with circles = M3G; purple with triangles = M6G; green with squares = codeine, and pink with diamonds = C6G

is no or very little gastrointestinal secretion.²⁰ In accordance, intestinal tracer uptake of i.v. ¹¹C-metformin has been reported to be minor.²²

Surprisingly, the CL_{total} after i.v. metformin was lower than the CL_{renal} after oral metformin both without and with codeine, although at least theoretically they should be identical (Table 2). In one of our previous studies, we administered ¹¹C-metformin intravenously and found that most of the dose was recovered in the kidneys, ureters, and in the urine bladder within ~ 10 min after injection.²² Further, the plasma concentration of metformin was much higher after i.v. compared with oral administration (Figure 1). Hence, the drug transporters in the proximal renal tubules that are responsible for most of the renal excretion of metformin¹ becomes saturated, and that reduces the clearance after i.v. administration. The same mechanism probably also explains why the absorption fraction (Equation 4) after i.v. metformin was lower than the F_{oral} (Equation 5; Table 2) based on the urinary recovery of metformin after oral administration.

The absorption fraction of metformin reported here is lower than that reported previously in two small studies ($F = \sim 50\text{--}60\%$),^{19,20} although in line with the results of a third study.²¹ However, we are confident that our results, which are based on more precisely measured plasma concentrations and a much larger sample, are more realistic.

There was a trend that codeine reduced the V of i.v. metformin and accordingly the $T_{1/2}$ (Equation 1). The results concerning V was, however, not statistically significant after removing two outliers and the clinical impact of this small change in V is questionable. In accordance, codeine did not have an impact on the oral V/F further supporting that codeine has no to a minimal effect on metformin V. Our results indicated that the intestinal absorption of metformin becomes delayed with ~ 1 h with concomitant codeine intake (Figure 1 and Table 2), although this did not reach statistical significance ($p = 0.06$). Because none of the volunteers had any of the common four reduced-function variants in the *OCT1* gene, we hypothesize that this is caused by

TABLE 3 The impact of 1 gram of orally or 0.5 gram of intravenously administered metformin on the steady-state pharmacokinetics of 25 mg of codeine in 16 healthy volunteers

	Single dose codeine before oral metformin	Steady-state codeine with oral metformin	GMR (95% CI)	Single dose codeine before i.v. metformin	Steady-state codeine with i.v. metformin	GMR (95% CI)
CL/F_{codeine}	^a 120 (82–149)	^b 107 (94–133) <i>n</i> = 15		^a 124 (91–144)	^b 110 (88–133) <i>n</i> = 16	
$T_{1/2\text{-codeine}}$	2 (1.9–2.8)	2.4 (2–3.2) <i>n</i> = 15		2.1 (1.9–2.5)	2.6 (2.1–3.0) * <i>n</i> = 16	
$AUC_{\text{codeine}}^{\text{ng}\cdot\text{h}}_{\text{mL}}$	^a 146 (118–213)	^b 164 (132–188) <i>n</i> = 15	1.02 (0.92–1.11)	^a 142 (122–193)	^b 159 (134–200) <i>n</i> = 16	1.07 (0.98–1.18)
$AUC_{\text{C6G}}^{\text{ng}\cdot\text{h}}_{\text{mL}}$	^a 2940 (2530–3330)	^b 3100 (2800–3600) <i>n</i> = 15	1.07 (0.99–1.16)	^a 2900 (2800–3300)	^b 3100 (2700–3600) <i>n</i> = 16	1.08 (0.99–1.17)
$AUC_{\text{morphine}}^{\text{ng}\cdot\text{h}}_{\text{mL}}$	^a 6.2 (4.3–7.4)	^b 7.1 (4.5–9.3) <i>n</i> = 12	1.21 (1.00–1.46)	^a 4.5 (3.5–7.5)	^b 6.4 (4.4–10.1) * <i>n</i> = 14	1.28 (1.09–1.49)
$AUC_{\text{M3G}}^{\text{ng}\cdot\text{h}}_{\text{mL}}$	^a 160 (126–245)	^b 202 (150–284) * <i>n</i> = 14	1.31 (1.17–1.46)	^a 155 (136–218)	^b 218 (166–297) * <i>n</i> = 14	1.34 (1.22–1.46)
$AUC_{\text{M6G}}^{\text{ng}\cdot\text{h}}_{\text{mL}}$	^a 32 (23–63)	^b 39 (32–63) * <i>n</i> = 15	1.27 (1.15–1.39)	^a 31 (26–48)	^b 46 (31–64) * <i>n</i> = 16	1.30 (1.18–1.40)

Data is presented as medians with the 25th–75th interquartile range.

Abbreviations: AUC, area under the plasma concentration time-curve; C6G, codeine-6-glucuronide; CI, confidence interval; CL/F, oral clearance; GMR, geometric mean ratio; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; $T_{1/2}$, terminal half-life.

Volunteers where it was not possible to fit an acceptable regression line to the terminal slope of the log plasma concentration or volunteers with extrapolated AUC >40% were not included in the statistical analyses.

N, Number of volunteers in the analyze.

* *P* value < 0.05.

^aUsing AUC_{0–∞} after a single dose of codeine.

^bUsing AUC_{0–6h} in steady-state together with oral or i.v. metformin.

inhibition of OCT1 at the gut level by codeine. A competing explanation could be codeine's negative effect on gastric motility and emptying,²³ which is known to impact the oral absorption of drugs.²⁴ The clinical impact of this small change in T_{max} is questionable and most likely does not explain the early discontinuation of metformin.

As previously observed,¹² the plasma concentrations of codeine and metabolites were high before ingestion of the last dose of codeine in steady-state, probably due to a deep compartment. The plasma concentration of morphine, M3G, and M6G seems to additively increase with increasing plasma concentrations of metformin (Figure 2, Table 3) indicating competitive inhibition of transporter-mediated uptake, although this was not statistically significant. Morphine and M6G have been suggested to be secreted in the urine²⁵ and morphine is also an OCT2 substrate.²⁶ Inhibition of the OCT2 transporter by metformin seems unlikely as the $I_{\text{maxunbound}}/IC_{50}$ value was well below 0.1. This is based on an IC_{50} value for MPP and not morphine, which is why inhibition by metformin cannot be ruled out. Placebo-controlled trials have reported an increased risk of adverse events in CYP2D6 normal but not in poor metabolizers after codeine intake,²⁷ most likely related to a higher morphine concentration in the former compared to the latter. Hence, the small amount of morphine and M6G produced is of importance for both effect and the risk of side effects, which is why small changes in the plasma concentrations as observed in this study seems to be of clinical importance. It is important to recognize that our study was not powered to detect changes in codeine pharmacokinetics, and median extrapolated AUC was greater than 20% for all of codeine's metabolites, which is a clear limitation in this study.

Pharmacodynamics

Despite standardized meals, plasma glucose had a higher peak following i.v. metformin compared to the oral administration after breakfast ($T = 4\text{h}$, $p = 0.005$). Animal studies have reported that oral metformin acutely lowers blood glucose probably by inhibiting intestinal glucose transport,²⁸ and similar results have been observed in patients with T2D, although only tested after a few days of treatment.²⁹

Median plasma lactate concentrations remained within the normal range at all visits (<2.0 mmol/L). The high plasma concentrations observed after an i.v. bolus of metformin does not result in higher levels of plasma lactate compared to oral administration of metformin. This is in agreement with previous research.²¹

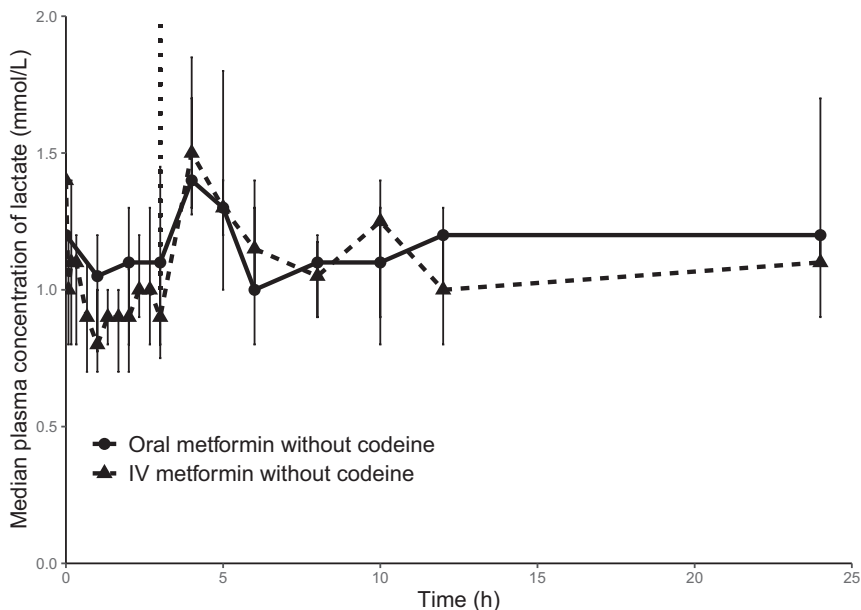


FIGURE 3 The median plasma concentration of lactate in 13 healthy volunteers presented as medians with the 25th–75th interquartile range. Statistical analysis on area under the plasma concentration time-curve from zero to 24 h (AUC_{0-24h}) and AUC_{0-12h} is based on 13 healthy subjects, whereas AUC_{0-3h} is based on 10 healthy subjects, due to missing 3-h blood samples and to only include the same volunteers as was used in the 24-h calculation. The lactate AUC_{0-24h} was significantly larger after oral compared to i.v. administration of metformin without codeine ($p = 0.047$). No significant difference was observed for the AUC_{0-12h} or AUC_{0-3h} . For the lactate and glucose calculations, volunteers who were missing the 24-h blood sample were excluded from the AUC calculations and statistical analysis. This was the case for three volunteers, whereas two others were missing all lactate and glucose blood samples from the i.v. metformin visit. Plasma lactate concentrations greater than 4 mmol/l was treated as outliers (probably due to too slow handling of the blood samples) and was left out of statistical analysis. This was the case for five blood samples

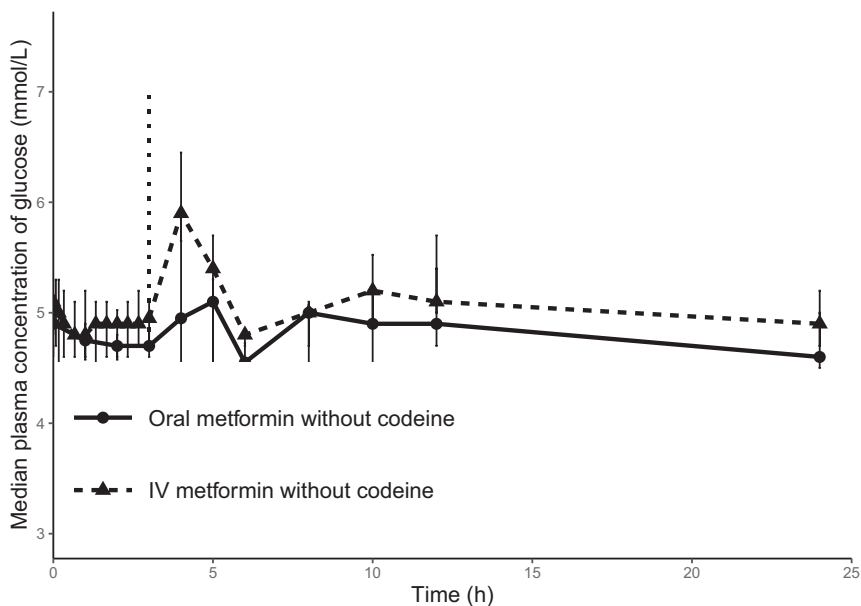


FIGURE 4 The median plasma concentration of glucose in 13 healthy volunteers presented as medians with the 25th–75th interquartile range. Statistical analysis on area under the plasma concentration time-curve from zero to 24 h (AUC_{0-24h}) and AUC_{0-12h} is based on 13 healthy subjects, whereas AUC_{0-3h} is based on 10 healthy subjects, due to missing 3-h blood samples and to only include the same volunteers as was used in the 24-h calculation. The glucose AUC_{0-24h} was significantly smaller in the oral compared to intravenous visit ($p = 0.03$). No significant difference was observed for the AUC_{0-12h} or AUC_{0-3h} . For the lactate and glucose calculations, volunteers who were missing the 24-h blood sample were excluded from the AUC calculations and statistical analysis. This was the case for three volunteers, whereas two others were missing all lactate and glucose blood samples from the i.v. metformin visit. Plasma lactate concentrations greater than 4 mmol/L was treated as outliers (probably due to too slow handling of the blood samples) and was left out of statistical analysis. This was the case for five blood samples

Intravenous metformin is quickly taken up by the liver,²² whereas the uptake in the intestine is sparse.²² The fact that the resulting higher plasma metformin concentrations did not cause a higher increase in lactate compared to oral intake of metformin seems to indirectly support the hypothesis that oral metformin mainly acts on the intestine,³⁰ although it is important to recognize that our study was not powered to detect pharmacodynamic changes.

The rise in plasma lactate observed at 4 h after oral and i.v. metformin can be explained by non-oxidative glycolysis seen in the postprandial phase in healthy subjects.^{31,32} The metformin-induced increase in systemic levels of plasma lactate is subtle in healthy volunteers even after 7 days of treatment.³³ We used a single dose of metformin and it only had a few hours to act before plasma lactate rose as part of the normal postprandial phase, which is why we cannot conclude that a single dose of oral metformin will cause an increase in systemic levels of plasma lactate.³² An ongoing trial (no. 2017-001132-19) has, however, recently provided evidence that the plasma lactate concentration increases in the hepatic portal vein in humans after oral metformin, which further supports the importance of the intestine for metformin mechanism of action.

CONCLUSION

This study shows that codeine does not affect the absorption fraction of metformin. Concomitant oral and i.v. metformin increased the plasma levels of morphine, M3G, and M6G. These small pharmacokinetic changes may well contribute to an increased risk of early discontinuation of metformin. Hence, a clinically relevant DDI between metformin and codeine seems plausible.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

K.B., T.B.S., M.M.H.C., T.K.B., and P.D. designed the research. I.K., T.B.S., M.M.H.C., T.K.B., K.H., P.D., and K.B. wrote the manuscript. I.K. and A.N.N. performed the research. I.K. analyzed the data. F.N. contributed new analytical tools.

DISCLAIMER

As an Editor-in-Training of *Clinical and Translational Science* Tore Bjerregaard Stage was not involved in the review or decision process for this paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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