Trimethoprim–metformin interaction and its genetic modulation by OCT2 and MATE1 transporters

Barbara Grün^{1*†}, Michael K. Kiessling^{1*}, Jürgen Burhenne¹,
Klaus-Dieter Riedel¹, Johanna Weiss¹, Geraldine Rauch²,
Walter E. Haefeli¹ & David Czock¹

¹Department of Clinical Pharmacology and Pharmacoepidemiology, University Hospital Heidelberg
and ²Institute of Medical Biometry and Informatics, University of Heidelberg, 69120 Heidelberg,
Germany

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Metformin is eliminated largely by renal secretion involving organic cation transporter 2 (OCT2) and the multidrug and toxin extrusion 1 (MATE1) transporter.
- The commonly used antibiotic trimethoprim inhibits OCT and MATE *in vitro*.
- The extent of a drug–drug interaction in an individual may depend on genetic polymorphisms.

WHAT THIS STUDY ADDS

- Trimethoprim inhibits metformin elimination in healthy volunteers and, on average, increases metformin exposure by 37%.
- An interaction was not observed in volunteers polymorphic for both OCT2 and MATE1.
- The extent of inhibition by trimethoprim was moderate, but might become clinically relevant in patients with borderline renal function or high-dose metformin.

Correspondence

PD Dr med. David Czock, Department of Clinical Pharmacology and Pharmacoepidemiology, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany.
Tel.: +49 6221 56 8740
Fax: +49 6221 56 4642
E-mail:
david.czock@med.uni-heidelberg.de

*B.G. and M.K.K. contributed equally to the study.

†Present address: Thorax Clinic Heidelberg, Department of Oncology, University Hospital Heidelberg, 69126 Heidelberg, Germany.

Part of this work was presented in abstract form at the ASCPT 2012 Annual Meeting (National Harbor, MD, USA, 12–17 March).

Keywords

drug interaction, genetic polymorphism, metformin, pharmacokinetics, trimethoprim

Received

30 May 2012

Accepted

22 December 2012

Accepted Article Published Online

11 January 2013

AIMS

Metformin pharmacokinetics depends on the presence and activity of membrane-bound drug transporters and may be affected by transport inhibitors. The aim of this study was to investigate the effects of trimethoprim on metformin pharmacokinetics and genetic modulation by organic cation transporter 2 (OCT2) and multidrug and toxin extrusion 1 (MATE1) polymorphisms.

METHODS

Twenty-four healthy volunteers received metformin 500 mg three times daily for 10 days and trimethoprim 200 mg twice daily from day 5 to 10. Effects of trimethoprim on steady-state metformin pharmacokinetics were analysed.

RESULTS

In the population as a whole, trimethoprim significantly reduced the apparent systemic metformin clearance (CL/F) from 74 to 54 l h⁻¹ and renal metformin clearance from 31 to 21 l h⁻¹, and prolonged half-life from 2.7 to 3.6 h (all $P < 0.01$). This resulted in an increase in the maximal plasma concentration by 38% and in the area under the plasma concentration–time curve by 37%. In volunteers polymorphic for both OCT2 and MATE1, trimethoprim had no relevant inhibitory effects on metformin kinetics. Trimethoprim was associated with a decrease in creatinine clearance from 133 to 106 ml min⁻¹ ($P < 0.01$) and an increase in plasma lactate from 0.94 to 1.2 mmol l⁻¹ ($P = 0.016$).

CONCLUSIONS

The extent of inhibition by trimethoprim was moderate, but might be clinically relevant in patients with borderline renal function or high-dose metformin.

Introduction

As a highly polar drug, the antidiabetic metformin crosses cell membranes poorly, and its pharmacokinetics are thus largely determined by the presence and activity of membrane-bound drug transporters. Such transporters facilitate absorption from the gut, distribution into liver and muscle cells, and renal elimination, which occurs mainly through active tubular secretion. Substantial transport activity has been shown for organic cation transporters (OCTs), multidrug and toxin extrusion transporters (MATEs) and the plasma membrane monoamine transporter (PMAT; Figure 1) [1–9]. In addition, paracellular absorption may be involved [10].

The impact of transport inhibitors on metformin exposure and clearance supports the crucial role of active metformin transport as a determinant of metformin disposition. *In vitro*, OCT and MATE were inhibited by cimetidine, trimethoprim and pyrimethamine [9, 11–15] and *in vivo*, metformin elimination was reduced by cimetidine [16, 17] and pyrimethamine [18], probably reflecting inhibition of renal tubular secretion through OCT2, MATE1, MATE2-K, or a combination thereof. The clinical relevance of these findings is unclear. A possible relationship between cimetidine and metformin-associated lactic acidosis was suspected in a case report in which short-term cimetidine was implied as a perpetrator [19]. Thus, other commonly used drugs potentially inhibiting metformin elimination, such as tri-

methoprim, an antibiotic frequently used for treating urinary tract infections, should be studied in humans.

In general, the extent of drug–drug interactions differs considerably between individuals. The reasons for this variability may include the effects of genetic polymorphisms. For cimetidine, lower or absent effects on metformin kinetics were observed in healthy Asian volunteers with the *OCT2* rs316019 single-nucleotide polymorphism (SNP) [16]. This finding is supported by *in vitro* studies in which higher cimetidine concentrations were required to inhibit metformin uptake into cells carrying the respective SNP [14].

We aimed to test the hypothesis that the OCT/MATE-inhibitor trimethoprim affects metformin pharmacokinetics *in vivo* and to evaluate the effects of selected functional *OCT2* and *MATE1* polymorphisms on the trimethoprim–metformin interaction.

Methods

Materials

Metformin tablets were obtained from Merck Pharma GmbH, Darmstadt, Germany (Glucophage® 500 mg) and trimethoprim tablets from Infectopharm Arzneimittel und Consilium GmbH, Heppenheim, Germany (Infectotrimet® 200 mg). Metformin and D₆-metformin (internal standard) reference compounds were purchased from Toronto

Figure 1

Schematic diagram of metformin transport within the body. Abbreviations are as follows: MATE, multidrug and toxin extrusion transporter; OCT, organic cation transporter; and PMAT, plasma membrane monoamine transporter. Missing arrowheads indicate unclear direction of transport. Question marks indicate fragmentary evidence

Research Chemicals (TRC, Toronto, Ontario, Canada). All other reagents and chemicals were of analytical grade and commercially obtained from local suppliers.

Study participants

Twenty-four volunteers (22 Caucasian, one African and one half-Korean) were enrolled in the study after providing written informed consent. Inclusion criteria were an age of 18–60 years, a good state of health (as defined by medical history, physical examination, electrocardiogram and routine laboratory analyses), an estimated creatinine clearance $> 60 \text{ ml min}^{-1}$ (Cockcroft & Gault equation), and known genotype for the selected *MATE1* and *OCT2* polymorphisms. Exclusion criteria were any regular drug treatment within the last 2 months (with the exception of oral contraceptives in female volunteers and L-thyroxine), any intake of substances known to inhibit or induce drug metabolizing enzymes or drug transporters, regular smoking, alcohol abuse, positive drug screening or known drug abuse, and known or planned pregnancy or breast feeding.

Average values for age, bodyweight and estimated creatinine clearance ($CL_{\text{crea,CG}}$) were comparable between subgroups. One female participant dropped out due to gastrointestinal adverse events after starting metformin and was replaced subsequently. In one stratum, only five of six planned volunteers could be recruited owing to the infrequent combination of the genetic polymorphisms of interest (Table 1).

Study design

This was an open-label, single-centre, single-sequence study. Volunteers were assigned to four strata according to the status of two common SNPs (*MATE1*, rs2289669; and *OCT2*, rs316019), as follows: stratum 1, *MATE1* and *OCT2* both homozygous wild-type (wt; *wtMATE1/wtOCT2*); stratum 2, polymorphic (poly) *MATE1* (homozygous for the A-allele), homozygous wild-type *OCT2* (*polyMATE1/wtOCT2*); stratum 3, homozygous wild-type *MATE1*, polymorphic *OCT2* (heterozygous, GT; *wtMATE1/polyOCT2*); and stratum 4, homozygous *MATE1* for the A-allele and hetero-

zygous *OCT2* (GT; *polyMATE1/polyOCT2*). Only six of 357 screened volunteers were homozygous for the polymorphic *OCT2* (TT), but none of them qualified for inclusion or agreed to participate within the time frame of the study.

Metformin 500 mg was administered orally three times daily (08.00, 14.00 and 19.00 h) from study day 1 to study day 10. Trimethoprim 200 mg was administered orally twice daily (08.00 and 19.00 h) from the evening of study day 4 to study day 10. The pharmacokinetics were analysed on study days 4 and 10. On the days before pharmacokinetic evaluation, the drug administration times were 08.00 h, 16.00 h and midnight for metformin (study days 3 and 9) and 08.00 and 20.00 h for trimethoprim (study day 9) in order to achieve steady-state conditions. On study day 10, trimethoprim was given half an hour before administration of metformin. Volunteers fasted from midnight to noon, and standardized meals were served at noon and 16.00 h on study days. Methylxanthine-containing beverages were not allowed on study days 3, 4, 9 and 10. Alcohol, grapefruit juice and St John's wort were not allowed throughout the study.

The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg and the competent authority in Germany (EudraCT no. 2010-019890-14) and was registered in the German Clinical Trials Register (DRKS-ID: DRKS00000481). The study was conducted at the Department of Clinical Pharmacology and Pharmacoepidemiology (certified according to ISO9001:2008) in accordance with the standards of Good Clinical Practice (as defined in the ICH E6 Guideline for Good Clinical Practice), in agreement with the principles as stated in the current version of the Declaration of Helsinki and the specific legal requirements in Germany.

Sample collection

On study days 4 and 10, heparinized blood samples were taken before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 h after administration of metformin. Blood samples were immediately centrifuged at $3600g$ at 4°C for 10 min, and separated plasma was frozen and stored at -20°C until analysis. Additional samples were taken for quantification of creatinine,

Table 1

Demographics

Stratum (genotype)	<i>n</i>	Sex (male/female)	Age (years)	Height (m)	Bodyweight (kg)	$CL_{\text{crea,CG}}$ (ml min^{-1})
1 (<i>wtMATE1/wtOCT2</i>)	6	3/3	32 ± 10	1.75 ± 0.10	71.6 ± 12.2	109 ± 30
2 (<i>polyMATE1/wtOCT2</i>)	6	3/3	29 ± 6	1.72 ± 0.09	75.8 ± 17.6	122 ± 30
3 (<i>wtMATE1/polyOCT2</i>)	5	3/2	33 ± 15	1.71 ± 0.06	72.2 ± 11.1	105 ± 20
4 (<i>polyMATE1/polyOCT2</i>)	6	3/3	31 ± 10	1.76 ± 0.10	73.1 ± 14.0	104 ± 14
Overall	23	12/11	31 ± 10	1.73 ± 0.09	73.2 ± 13.2	110 ± 24

Data are shown as means $\pm$ SD. Abbreviations are as follows: $CL_{\text{crea,CG}}$, estimated creatinine clearance (Cockcroft & Gault); MATE, multidrug and toxin extrusion; OCT, organic cation transporter; poly, polymorphic; and wt, wild-type.

glucose, lactate, insulin and C-peptide. Creatinine was quantified in 0, 3 and 8 h samples.

Volunteers emptied their bladder directly before the 08.00 h metformin administration. All urine was collected 0–3, 3–6 and 6–8 h after dosing. The content of each container was stirred, the volume documented, and an aliquot of 10 ml frozen and stored at -20°C . In addition, at the end of a study day, all urine of a volunteer was mixed, the volume documented, and a sample frozen at -20°C until analysis.

Quantification of metformin in plasma and urine

Protein precipitation was applied to plasma samples by addition of acetonitrile after spiking with internal standard. An aliquot of the supernatant was diluted 1:4 with high-performance liquid chromatography (HPLC) eluent and transferred into HPLC vials. Urine samples were diluted 1:500 with HPLC eluent and spiked with internal standard. Aliquots of plasma and urine extracts were injected into the liquid chromatography–tandem mass spectrometry system, which consisted of a SIL-10ADvP autosampler, a LC-10ADvP pump (Shimadzu, Duisburg, Germany) and an API 365 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a Synergy Polar-RP column 150 mm $\times$ 2.5 mm (Phenomenex, Aschaffenburg, Germany) at 40°C . The isocratic HPLC eluent was 50% 5 mM ammonium acetate including 0.1% acetic acid and 50% acetonitrile at a flow rate of 0.35 ml min^{-1} . The tandem mass spectrometry transitions monitored in the positive ion mode for metformin and D_6 -metformin were $m/z\ 129.9 \rightarrow m/z\ 60.1$ and $m/z\ 135.9 \rightarrow m/z\ 60.1$. Nitrogen was used for collision-induced fragmentation. Data collection, peak integration and calculations were performed using Analyst V 1.4.2 software (Applied Biosystems). Peak area ratios were used for construction of calibration curves by $1/x$ weighted linear regression. The method was calibrated in the range of 5–5000 ng ml^{-1} , and quality control was performed at concentrations of 21.9, 1430 and 2970 ng ml^{-1} for plasma and urine samples. The method was validated according to the US Food and Drug Administration guidelines [20]. The metformin extraction recovery ranged from 93 to 116%. The lower limit of quantification was 5 ng ml^{-1} , and calibration was linear, with correlation coefficients $r^2 > 0.99$. The overall accuracy and precision (day to day and within day) were 97.0–105.1 and 0.9–10.6%, respectively, for plasma. For urine, the overall accuracy and precision were 96.2–102.9 and 4.0–6.5%, respectively.

Determination of MATE1 and OCT2 SNPs

The *MATE1* rs2289669 and the *OCT2* rs316019 SNPs were detected by melting curve analysis of polymerase chain reaction (PCR) products using the hybridization probes format on a LightCycler® 480 (Roche, Mannheim, Germany). All materials for real-time PCR were from Roche

Applied Science (Mannheim, Germany). Primers and hybridization probes were designed and synthesized by TIB MOLBIOL (Berlin, Germany). The following primers and probes were used: rs2289669 forward, 5'-CAAAGCCCAGTTTGTGCTAAG-3'; rs2289669 reverse, 5'-GCAGCCTCATGCCTCACAC-3'; rs2289669 sensor probe, 5'-GGAACCTCCACGCTACTGT-fluorescein; rs2289669 anchor probe, 5'-LC640-ACTGAGCCCCAGGTTACGATGC-PH-3'; rs316019 forward, 5'-AGAGTGAAAGCAATCTCAGAA TCAG-3'; rs316019 reverse, 5'-TGATAGCTGGACAGCCA ACT-3'; rs316019 sensor probe, 5'-LC640-TCACAGTTT CTCTGCCAA-PH-3'; and rs316019 anchor probe, 5'-TCCTCACTGGAGGTGGTTGCA-fluorescein-3'.

Quantification of biomarkers

Creatinine, glucose, lactate, insulin and C-peptide were measured in the accredited central laboratory of the University Hospital Heidelberg. Creatinine concentrations in plasma were quantified by a validated enzymatic method and in urine by a validated Yaffe method. Insulin resistance was estimated using the homeostasis model assessment equation for insulin resistance (HOMA-IR) [21].

Pharmacokinetic analysis

Steady-state metformin kinetics were analysed with standard noncompartmental equations. The area under the curve from 0 to 8 h (AUC_{0-8}) was calculated using the linear-trapezoidal method. The predose concentration (C_0), maximal concentration (C_{max}) and time of maximal concentration (t_{max}) were obtained directly from observed concentrations. The apparent systemic clearance after oral administration was calculated as $\text{CL}/F = \text{dose}/\text{AUC}_{0-8}$. The elimination rate constant (λ) was obtained by linear regression analysis of the log-transformed values of the concentration decline and the half-life calculated as $t_{1/2} = \ln(2)/\lambda$. The apparent volume of distribution after oral administration was calculated as $V_d/F = \text{CL}/F/\lambda$. The amount recovered in urine (A_e) was calculated by multiplying urinary concentration and volume, and the relative urinary recovery (U_R) was calculated as $A_{e,0-8}/\text{dose} \times 100$. Renal clearance was calculated as $\text{CL}_{\text{ren,metformin}} = A_e/\text{AUC}$, and creatinine clearance was calculated as $\text{CL}_{\text{ren,crea}} = A_{e,\text{crea}}/\text{AUC}_{\text{crea}}$, where AUC_{crea} was calculated from the 0, 3 and 8 h values using the linear-trapezoidal method.

Statistical analysis

Demographic parameters are reported as means $\pm$ SD. Differences between the four subgroups were analysed with one-way ANOVA.

Drug concentrations are shown as means $\pm$ SEM. Differences between concentrations at a specific time point were analysed with Student's two-sided *t*-test for paired data without adjustment for multiple comparisons.

Pharmacokinetic parameters were logarithmically transformed and deviations from normality analysed with the D'Agostino & Pearson omnibus normality test.

Trimethoprim-associated changes were analysed with Student's two-sided *t*-test for paired data or a two-sided Wilcoxon signed-rank test, as appropriate. Genotype-associated differences between subgroups were analysed with one-way ANOVA and Tukey's *post hoc* test or Kruskal–Wallis test and Dunn's *post hoc* test, as appropriate. Biomarker values were logarithmically transformed where indicated by the normality test and analysed with Student's two-sided *t*-test for paired data. A value of $P < 0.05$ was considered statistically significant. Average pharmacokinetic parameter values are reported as geometric means and 95% confidence intervals.

The sample size ($n = 6$ per stratum) was based on a power calculation for a Student's two-sided paired *t*-test, assuming an average metformin CL/F of 70 and 35 l h⁻¹ on study days 4 and 10, respectively, and a standard deviation for the difference between these days of 24 l h⁻¹ based on published data [17, 22, 23]. The predefined primary endpoint of this trial was the change in CL/F between study days 4 and 10 in stratum 1.

Software

Calculations were done with Microsoft® Excel 2003 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism Version 5.01 (GraphPad Software, San Diego, CA, USA).

Results

Effects of trimethoprim on metformin kinetics

Metformin plasma concentrations increased significantly during cotreatment with trimethoprim (Figure 2). The CL/F and $CL_{ren,metformin}$ were decreased to 73 and 68%, whereas $t_{1/2}$, AUC_{0-8} , C_0 , and C_{max} were increased to 133, 137, 133 and 138% (all $P < 0.01$). Values of V_d/F , t_{max} and U_R were unchanged in comparison to metformin monotherapy (Table 2).

When analysing the effects of trimethoprim on metformin kinetics within subgroups, a similar trend was observed for most parameters, but statistical significance was reached only in some cases, probably because the effect was lower than expected (Supplemental Tables S1 and S2). Also, the change in our predefined primary endpoint as used for power calculation, i.e. the trimethoprim-associated change in metformin CL/F in the *wtMATE1/wtOCT2* subgroup (stratum 1) was statistically not significant (CL/F 63.3 l h⁻¹ during metformin monotherapy, 52 l h⁻¹ during cotreatment with trimethoprim; $P = 0.097$).

Genetic modulation of the trimethoprim–metformin interaction

When comparing the effects of trimethoprim on metformin kinetics between subgroups, these effects appeared to be smaller or absent in the *polyMATE1/polyOCT2* subgroup (stratum 4) as indicated by visual

inspection of Figure 2 and the absence of statistically significant changes in this stratum.

Effects on biomarkers

Cotreatment with trimethoprim was associated with a statistically significant increase in plasma creatinine from 0.88 to 1.08 mg dl⁻¹, a decrease in $CL_{crea,CG}$ from 114 to 93 ml min⁻¹, a decrease in $CL_{ren,crea}$ from 133 to 106 ml min⁻¹, and an increase in lactate from 0.94 to 1.24 mmol l⁻¹ (Table 3). When analysing subgroups, a trend to lower glucose values was found in the *polyMATE1/polyOCT2* subgroup (stratum 4; $P = 0.056$). The increase in lactate was significant only in the *wtMATE1/polyOCT2* subgroup (stratum 3), and there was apparently no increase in lactate in the *polyMATE1/polyOCT2* subgroup (stratum 4; Supplemental Tables S3 and S4).

Genotype-associated differences were not found between estimated creatinine clearance or between measured 8 h creatinine clearance values (Supplemental Tables S3 and S4). Overall, measured creatinine clearance was significantly higher than estimated creatinine clearance ($P < 0.01$).

Safety and tolerability

Serious adverse events were not observed. One moderate adverse event (nausea and weakness) accompanied by mild loss of appetite and vomiting occurred in one female participant from the *wtMATE1/wtOCT2* subgroup (stratum 1). Mild gastrointestinal adverse events (loss of appetite, nausea, gastrointestinal pain, diarrhoea or vomiting) occurred in 13 volunteers ($n = 4, 1, 3, 5$ in strata 1–4). Increased lactate (i.e. >1.6 mmol l⁻¹) occurred in 10 volunteers before, during or after the study. These increases could be explained by strenuous exercise and were transient in all cases.

Discussion

Metformin is usually well tolerated, but its use has been associated with lactic acidosis, probably facilitated by high metformin concentrations affecting mitochondrial energy metabolism [24, 25]. High metformin concentrations may be due to suicidal intoxication, which causes lactic acidosis also in patients with normal renal function [26, 27], and accidental intoxication when metformin administration is continued during acute renal failure [24, 27]. Systematic studies do not indicate a higher risk of lactic acidosis in patients treated with metformin [28]. However, such studies usually include well-defined patient populations. Thus, in patients with further risk factors, the risk of lactic acidosis could be higher [29]. Whether trimethoprim (or cotrimoxazole) is a risk factor has not been evaluated so far. Theoretically, this could be important because urinary tract infections were reported in 29% of patients presenting with lactic acidosis [24].

Figure 2

(A) Concentration-time profiles of metformin in healthy volunteers after oral administration of 500 mg three times daily (filled circles) and during cotreatment with trimethoprim (open circles). (B) Volunteers wild-type (wt) for *MATE1* and *OCT2*. (C) Volunteers with polymorphic (poly) *MATE1* (rs2289669) and wild-type *OCT2*. (D) Volunteers with wild-type *MATE1* and polymorphic *OCT2* (rs316019). (E) Volunteers with polymorphic *MATE1* and *OCT2*. Each point represents the mean $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ (comparison at the respective time point)

Table 2

Steady-state metformin pharmacokinetics after oral administration of 500 mg three times daily as monotherapy and during cotreatment with trimethoprim

Parameter	Metformin	Metformin + trimethoprim	P value
CL/F (l h ⁻¹)	74 (65–84)	54 (46–62)	<0.001*
CL_{ren} (l h ⁻¹)	31 (26–37)	21 (17–27)	0.003†
V_d/F (l)	286 (245–333)	279 (229–339)	0.809*
$t_{1/2}$ (h)	2.7 (2.5–2.9)	3.6 (3.2–4.1)	<0.001*
AUC (h µg ml ⁻¹)	6.8 (6.0–7.7)	9.3 (8.0–10.7)	<0.001*
C_0 (µg ml ⁻¹)	0.54 (0.45–0.65)	0.72 (0.59–0.87)	<0.001*
C_{max} (µg ml ⁻¹)	1.3 (1.1–1.5)	1.8 (1.6–2.1)	<0.001*
t_{max} (h)	1.5 (0.5–4.0)	2.0 (0.6–3.5)	0.486*
U_R (%)	42 (34–51)	40 (33–48)	0.207†

Data are shown as geometric means and 95% confidence intervals. The values for t_{max} are shown as the medians and ranges. Abbreviations are as follows: AUC, area under the curve; C_0 , predose concentration; CL/F , apparent clearance after oral administration; CL_{ren} , renal clearance; C_{max} , maximal concentration; $t_{1/2}$, half-life; t_{max} , time of maximal concentration; U_R , urinary recovery; and V_d/F , apparent volume of distribution after oral administration. *Student's *t*-test. †Wilcoxon signed-rank test.

Table 3

Biomarker after oral administration of 500 mg three times daily as monotherapy and during cotreatment with trimethoprim

Parameter	Metformin	Metformin + trimethoprim	P value
Creatinine (mg dl ⁻¹)	0.88 (0.82–0.95)	1.08 (1.01–1.14)	<0.001*
$CL_{crea,CG}$ (ml min ⁻¹)	114 (103–125)	93 (84–104)	<0.001*
$CL_{ren,crea}$ (ml min ⁻¹)	133 (119–149)	106 (94–120)	<0.001*
Glucose (mg dl ⁻¹)	89 (86–92)	89 (85–92)	0.762*
Insulin (mU l ⁻¹)	6.8 (5.7–8.1)	7.2 (5.7–9.2)	0.441†
C-peptide (ng ml ⁻¹)	1.4 (1.2–1.6)	1.5 (1.3–1.7)	0.185*
HOMA-IR	1.5 (1.3–1.8)	1.6 (1.2–2.0)	0.521†
Lactate (mmol l ⁻¹)	0.94 (0.79–1.10)	1.24 (1.07–1.44)	0.016†

Data are shown as geometric means and 95% confidence intervals. The *P* values are from Student's *t*-test using untransformed (*) or logarithmically transformed data (†). Abbreviations are as follows: $CL_{crea,CG}$, estimated creatinine clearance (Cockcroft & Gault); $CL_{ren,crea}$, measured 8 h creatinine clearance; and HOMA-IR, estimated insulin resistance (homeostasis model assessment equation for insulin resistance).

Our present study confirms expectations of increased metformin concentrations during treatment with trimethoprim; the extent of the interaction was in the expected range and can be explained by inhibition of renal metformin elimination. Based on the reported cimetidine-associated reduction in metformin renal clearance to 44% within the first 3 h, in comparison to metformin monotherapy [17], the relatively low cimetidine dose in that earlier study (400 mg every 12 h) in comparison to cimetidine doses in studies aiming at maximal suppression of creatinine secretion (800 mg every 8–12 h) [30–32], the potent trimethoprim-associated inhibition of OCT and MATE *in vitro* [11, 14, 15], the trimethoprim-associated

reduction in creatinine clearance in humans [33–37] and the longer half-life of trimethoprim in comparison to cimetidine (11 vs. 2 h [38, 39]), we expected a reduction in systemic metformin clearance up to 50% and a doubling of metformin concentrations. However, the observed metformin clearance was reduced to 73%, and average concentrations were increased only to 137% in comparison to metformin monotherapy. Likewise, pyrimethamine (half-life 140 h [40]) increased the 12 h metformin AUC of a single 250 mg metformin dose to 135% [18]. One explanation might be incomplete inhibition of renal drug transporters, which would be in agreement with the following considerations. Assuming a trimethoprim systemic clearance of 8.8 l h⁻¹, an unbound fraction of 0.5 [41] and near-complete absorption, an average unbound trimethoprim concentration of 3.3 µmol l⁻¹ is predicted for trimethoprim 200 mg twice daily. Assuming a metformin systemic clearance of 31 l h⁻¹ [41], exclusive renal elimination of metformin and a glomerular filtration rate of 120 ml min⁻¹, a secreted fraction of 0.77 is predicted. Using the Rowland and Matin drug–drug interaction equation [42] as modified for excretory transport [43], inhibitory concentrations $IC_{50} = 1327$ µmol l⁻¹ for OCT2 [14] and $IC_{50} = 6.2$ µmol l⁻¹ for MATE1 [44], and assuming linear pharmacokinetics, intracellular concentrations similar to plasma concentrations and unchanged metformin absorption, a negligible effect due to OCT2 inhibition and a 1.36-fold increase due to MATE1 inhibition would be predicted, which is consistent with the observed values.

Our finding of a trimethoprim-associated increase in metformin concentrations might be relevant for patients with diabetes mellitus, despite the mild increase in healthy participants. First, the trimethoprim-associated increase in metformin concentrations could add to already high concentrations, e.g. due to borderline renal function. Second, more pronounced effects of cimetidine and trimethoprim on serum creatinine have been observed in patients with impaired renal function [32, 37], indicating a higher fractional clearance by secretion when glomerular filtration decreases. Third, as a consequence of reduced metformin efflux out of hepatocytes the intrahepatic metformin concentrations might increase more than plasma concentrations and facilitate lactic acidosis, as indicated by studies with *Mate1* knockout mice [45, 46].

The extent of the trimethoprim–metformin interaction appears to depend on genotype, as indicated by the absence of significant inhibition by trimethoprim in the *polyMATE1/polyOCT2* subgroup (stratum 4) as opposed to all other strata. However, study design and number of volunteers in the present study did not allow for statistically based conclusions. Notably, in volunteers polymorphic for OCT2 and wild-type for MATE1 (stratum 3) an interaction was observed. In an earlier report in Chinese volunteers polymorphic for OCT2, no interaction with metformin was observed using cimetidine as an inhibitor [16], but MATE1 polymorphisms were not analysed in that study.

A number of limitations are worth mentioning. First, our study followed a sequential study design. While this design is adequate for assessment of metformin kinetics, metformin pharmacodynamics are difficult to interpret because we cannot distinguish trimethoprim effects from enhanced effects due to prolonged metformin therapy. Second, only one dose level was analysed in healthy volunteers. Trimethoprim 200 mg twice daily represents the highest dose approved for monotherapy and is similar to the 160 mg twice daily commonly used with cotrimoxazole. Metformin 500 mg three times daily is a common dose and 50% of the maximal recommended dose in patients. Thus, we cannot show whether or not the degree of the trimethoprim–metformin interaction is modulated by drug dose or renal function. Third, other transporters potentially affecting metformin disposition (OCT1, OCT3, MATE2-K or PMAT) were not analysed for genetic polymorphisms. Finally, trimethoprim concentrations were not quantified, and it is thus unknown whether trimethoprim exposure differs between subgroups.

In conclusion, trimethoprim significantly reduced metformin elimination and increased metformin exposure, and this effect appears to depend on the genotype. Overall, this interaction was moderate, suggesting that it is only of potential relevance if high metformin doses are administered and/or in patients with borderline renal function.

Conflict of Interest

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

We thank Marlies Stützle-Schnetz, Brigitte Tayrouz, Magdalena Longo and Jutta Kocher for their skilful technical and consultative assistance and Christoph Markert for clinical trial monitoring.

The development of the analytical method was in part supported by the Federal Ministry of Education and Research (BMBF), grant no. 01ET0718.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1

Steady-state metformin pharmacokinetics after oral administration of 500 mg three times daily as monotherapy according to subgroup

Table S2

Steady-state metformin pharmacokinetics after oral administration of 500 mg three times daily during cotreatment with trimethoprim according to subgroup

Table S3

Biomarker during metformin monotherapy according to subgroup

Table S4

Biomarker during cotreatment with trimethoprim according to subgroup