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ARTICLE

Dose-dependent induction of CYP3A activity by St. John's wort alone and in combination with rifampin

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Abstract

The dose dependence of the effect of enzyme inducers and the effect of the combined administration of two inducers that exert their effect via the same induction pathway (pregnane X receptor) have not been well studied. Using oral midazolam microdoses (30 μg), we have investigated CYP3A4 induction by St. John's wort (SJW) in 11 healthy volunteers using low (300 mg/day containing 7.48 mg hyperforin), therapeutic (900 mg/day), and supratherapeutic doses of SJW (1800 mg/day) for 14 days. SJW was then co-administered with rifampin (600 mg/day) for a further 7 days to evaluate the effect of the combined administration of two inducers. In addition, intravenous midazolam microdoses (10 μg) were administered before SJW, at SJW 1800 mg/day, and during administration of the two inducers to assess the hepatic contribution to total induction (semi-simultaneous administration). Administration of SJW increased oral midazolam clearance 1.96-fold (300 mg/day), 3.86-fold (900 mg/day), and 5.62-fold (1800 mg/day), and 17.5-fold after the addition of rifampin. Concurrently, the clearance of intravenous midazolam increased 2.05-fold (1800 mg/day) and 2.93-fold (SJW + rifampin). These results show that rifampin significantly enhances the induction of the highest SJW doses both hepatically and overall and suggest that these metabolic effects occur predominantly in the gut. These findings also suggest that in drug interactions involving strong and moderate enzyme inducers, the perpetrator effects of the strong inducer are decisive for the interaction.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

An increasing number of drugs that can activate the pregnane X receptor (PXR) are on the market. These drugs have the potential to increase the expression of important pharmacokinetic targets such as cytochrome P450 (CYP) isozymes and drug transporters, thereby substantially altering the clearance of co-administered substrates. Limited evidence in humans suggests that PXR

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induction is dose-dependent and that the extent of induction can vary depending on the inducer. Little is known about the co-administration of two inducers acting through the same pathway.

WHAT QUESTION DID THIS STUDY ADDRESS?

Our aim was to characterize the dose-dependent effect of St. John's wort (SJW) on oral clearance of microdosed midazolam as a marker for CYP3A4 activity and to investigate the effect of adding a strong inducer (rifampin) to the highest SJW dose. In addition, we assessed the contribution of hepatic clearance to total midazolam clearance at the highest SJW dose and during its combination with rifampin.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This is the first trial thoroughly characterizing the dose dependence of CYP3A4 induction by SJW in humans. It revealed that the clearance increases occur dose-dependently, predominantly extrahepatically, and likely at the level of the gut. The trial also revealed that the co-administration of rifampin further increased midazolam clearance to values known from trials that analyzed rifampin induction without SJW.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The findings of this trial indicate that if a moderate inducer (SJW) and a strong inducer (rifampin) acting via the same PXR pathway are combined, the perpetrator effects of the stronger inducer dominate.

INTRODUCTION

Phenotyping the in vivo activity of drug-metabolizing enzymes such as cytochrome P450 (CYP) isozymes is an important technique that enables (a) the screening and quantitative assessment of the potential of a drug to induce pharmacokinetic drug interactions during drug development, (b) the investigation of the effects of medical conditions on the drug elimination capacity of the human body, and (c) the development of patient-specific strategies for precision dosing of drugs with a narrow therapeutic index whose clearance (CL) is CYP-dependent.[1] Hence, the development and refinement of techniques that facilitate phenotyping, such as limited-sampling strategies (LSS), use of endogenous biomarkers, phenotyping cocktails, and microdosing, are important as they allow to phenotype more vulnerable populations more often and in a more economical way.[1–3]

Midazolam is the paradigm marker substrate recommended by the European Medicines Agency and the US Food and Drug Administration (FDA) for measuring the activity of the CYP3A isozyme.[4,5] Microdoses as low as 10 ng can be used to characterize pharmacokinetic drug interactions.[6,7] Midazolam microdoses can be administered orally to measure apparent oral CL (CL/*F*) or intravenously (i.v.) to measure systemic CL (CL_{sys}).[6,7] We have used these techniques in the past for repetitive phenotyping to assess the effect of time on complex drug interactions[7–9] or to phenotype critically ill patients suffering from hematological tumors.[10] Up to now, these trials investigated the effect of CYP inhibition. We have recently characterized the time course of induction by rifampin with a continuous infusion of microdosed midazolam.[7] However, with the more common oral or i.v. bolus administration of midazolam microdoses, the effects of CYP3A induction on the pharmacokinetics of midazolam are still unclear.

Depending on the preparation, St. John's wort (SJW) is a strong inducer of CYP3A activity, which can lead to severe drug–drug interactions,[11] for which its active ingredient hyperforin is mainly responsible.[12] Similarly, also rifampin is a strong inducer, and both hyperforin and rifampin act by binding to the intracellular pregnane X receptor (PXR).[13] Mechanistically, the induction of CYP3A4 is the result of the binding of a small molecule to the ligand-binding pocket of PXR, which in turn leads to the translocation of PXR from the cytoplasm to the nucleus where two PXR form a heterotetramer with two retinoic X receptors (RXR). Binding of the PXR/RXR heterotetramer to a DNA-binding domain leads to the recruitment cascade of co-factors that enhances the transcriptional activity and results in increased CYP3A4 gene expression.[14] Although both hyperforin (molecular weight: 536.8 Da)[15] and the larger rifampin

(822.9 Da)[16] bind to PXR, their binding properties differ and the binding of the larger rifampin can displace the walls of the PXR ligand pocket.[13,17] However, rifampin's binding to the PXR binding pocket is more than 20-fold weaker (EC_{50} 0.71 μM) than the binding of hyperforin (0.032 μM),[18] suggesting that rifampin may not further enhance induction in the presence of hyperforin.

In this clinical trial in healthy volunteers, we used both oral and i.v. midazolam microdoses in a semi-simultaneous fashion[19,20] to evaluate the dose-dependent effect of SJW on CYP3A activity and its modification by a second paradigm inducer rifampin.

MATERIALS AND METHODS

Regulatory

The study protocol was approved by the Competent Authority in Germany (BfArM, clinical trial registration: EudraCT No: 2013-004374-10 registered on Oct. 10, 2013) and the responsible Ethics Committee of the Medical Faculty of Heidelberg University. It was conducted at the DIN EN ISO9001-certified Early Clinical Trial Unit (KliPS) of Internal Medicine IX, Department of Clinical Pharmacology and Pharmacoepidemiology in accordance with the standards of Good Clinical Practice (as defined in the ICH E6 Guideline for Good Clinical Practice) and in agreement with the Declaration of Helsinki and the specific legal requirements in Germany.

Study participants

All study participants gave their written informed consent for the study and for the storage of DNA in a biobank for the genotyping of metabolic enzymes before any study measures were carried out. They were physically and mentally healthy as confirmed by the medical history, a physical examination, a 12-lead electrocardiogram, and routine laboratory analyses including kidney and liver function tests, hematology, glucose, urinalysis, urinary screening for illicit drugs, and pregnancy testing (women of child-bearing potential). Female participants had to agree to use two highly effective contraceptive methods. After the conclusion of the trial, the participants were genotyped for CYP3A5 as previously described[6]; one carried *1/*3 alleles (active enzyme), nine were poor metabolizers (*3/*3), and of two participants no DNA was available.

Study conduct

The study was carried out as an open clinical trial with a fixed-sequence design without washout phases (Figure 1). The sequence consisted of a baseline assessment of CYP3A activity followed by CYP3A induction with 300 mg SJW (Jarsin®300, Casella-med, Cologne, Germany; average hyperforin content 7.48 mg/dose unit[21]) once daily for 14 days (SJW 300). The SJW dose was then increased to 3 × 300 mg daily for 14 days (SJW 900), followed by a further increase to 3 × 600 mg daily for 14 days (SJW 1800). This dose was then maintained for a further 7 days together with 600 mg rifampin once daily (Eremfat®, Riemser Arzneimittel AG, Greifswald, Germany). To assess total and hepatic CYP3A activity, a fixed-sequence approach with "semi-simultaneous" oral and subsequent i.v. administration of midazolam was carried out as described earlier,[20] but with microdoses instead of pharmacologically active doses. On three separate study days (baseline, SJW 1800, SJW 1800 + rifampin 600), participants received 30 μg of midazolam solution orally and 6 h later 10 μg of i.v. midazolam over 5 min (Dormicum®V 5 mg/5 mL solution for injection, Roche, Grenzach-Wyhlen, Germany). Oral medication was self-administered by the participants, except on days with pharmacokinetics, when all doses were

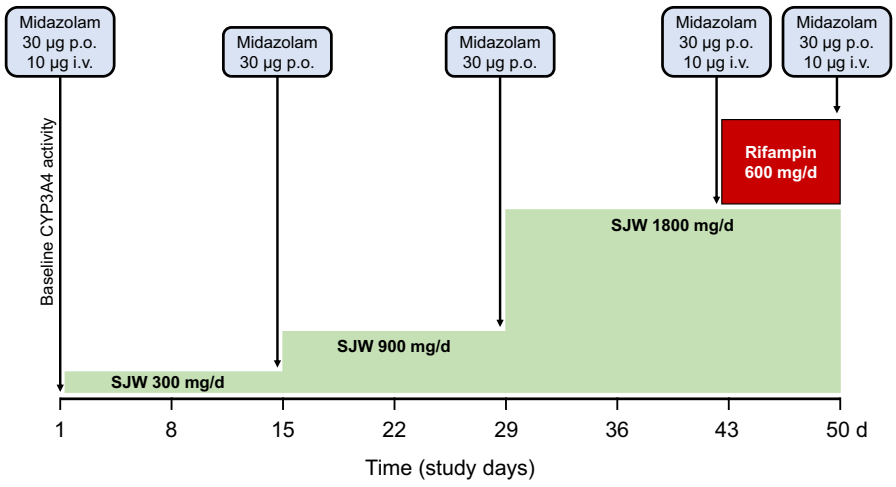

FIGURE 1 Study design with four consecutive induction phases using 300, 900, and 1800 mg St. John's wort (SJW, green) daily each for 14 days, and combining 1800 mg SJW daily with 600 mg rifampin (red) daily for 7 days. CYP3A activity was determined on five study days with the respective midazolam doses indicated.

administered on-site. Blood samples were taken before drug administration and at 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after oral administration of midazolam and at 15, 30, 40, 50 min and 1, 1.25, 1.5, 2, 3, 4, 6, and 8 h after the start of the i.v. administration of midazolam. On the study days of SJW 300 and SJW 900, only a 30-µg oral dose was administered and a LSS was used taking five blood samples (pre-dose, 2, 2.5, 3, and 4 h).[22] The blood samples were centrifuged at 2500*g* at 4°C, the samples were stored at −20°C until analysis.

Midazolam quantification

Midazolam concentrations in plasma were determined by a validated UPLC-MS/MS method as previously described.[23] The assay's lower limit of quantification was 0.37 pg/mL and the accuracy/precision values were 96%–99%/≤12.0%.

Pharmacokinetic calculations

Standard non-compartmental pharmacokinetic parameters of midazolam were calculated using PKanalix (Lixoft Inc, Antony, France)[24]: On days with full pharmacokinetic profiles, the maximum plasma concentration of midazolam (C_{max}) and the time to reach C_{max} (t_{max}) were taken directly from the measurements. The terminal elimination half-life ($t_{1/2}$) was calculated from the plasma concentration–time data up to 8 h after i.v. administration by determining ln 2/λ, where λ is the terminal slope calculated by linear regression of the time versus log concentration data. The area under the plasma concentration–time curve (AUC_{∞}) after oral administration was calculated using the linear-up log-down trapezoidal rule and the λ value of the i.v. administration was used to extrapolate the area from 6 h to infinity. The AUC_{∞} after i.v. administration was calculated as AUC from the start of i.v. administration minus the extrapolated AUC, attributable to the oral dose ($AUC_{6\text{-}\infty\ po}$). Similarly, for the definition of C_{max} after i.v. administration, the extrapolated concentration originating from the preceding oral administration was subtracted from the measured C_{max} value. CL/*F* and CL_{sys} were calculated using the appropriate AUCs. Bioavailability (*F*) was calculated on the basis of the AUC_{∞} of oral midazolam and the AUC_{∞} of i.v. midazolam minus $AUC_{6-\infty\ po}$. For the evaluation of the total CYP3A activity on all 5 study days, the midazolam metabolic CL (eCL_{met}) was estimated by the previously described LSS methodology using the AUC from 2 to 4 h ($AUC_{2\text{-}4}$) after oral administration.[22] To estimate the relative contribution of extrahepatic midazolam metabolism to total CL (eCL_{met}), we subtracted CL_{sys}, which is thought to primarily reflect hepatic CL, and expressed the geometric mean values of the resulting estimated intestinal CL as a percentage of eCL_{met}.

Statistics

Data are presented as geometric mean with 95% confidence intervals (95% CI) unless stated otherwise. Corrected for multiple comparisons using the Sidak test, a repeated measures ANOVA on log-transformed data was applied to test for differences between inducing substances and baseline. The relationship between SJW dose and CYP3A induction was analyzed using the classical dose–response equation with variable slope and the minimum (E_{min}) fixed to baseline CYP3A activity (Hill equation). The relationship between $AUC_{2\text{-}4}$ and AUC_{∞} of midazolam was analyzed using linear regression analysis. All statistical calculations were performed with Prism 9.4 (GraphPad Software, La Jolla, CA, USA), and a *p*-value <0.05 was considered significant.

RESULTS

Study participants

Twelve participants (six females) were included but one female participant dropped out during treatment with 1800 mg SJW due to adverse effects (allodynia, erythrodermia), an observation which was reported earlier.[25] Therefore, 11 participants (age: 26 ± 4 years; weight: 71 ± 19 kg; body mass index: 23.8 ± 5.3 kg m^2) completed the study.

Midazolam pharmacokinetics

Repeated administration of 1800 mg SJW substantially decreased midazolam exposure after oral and i.v. administration (Figure 2; Table 1) and enhanced midazolam CL 2.04-fold after i.v. and 4.62-fold after oral administration. Concurrently, also $t_{1/2}$ was reduced and the absolute *F* of midazolam decreased from 35.1% at baseline to 15.6%.

The addition of 600 mg rifampin to high SJW doses for 7 days further increased midazolam CL 1.43-fold i.v. (2.93-fold compared with baseline) and 3.06-fold orally (14.1-fold compared with baseline) (Table 1). The effect of the two inducers was several times more pronounced after oral administration of midazolam than after i.v. administration, with CL values being 1.43-fold higher after i.v. and 3.06-fold higher after oral administration.

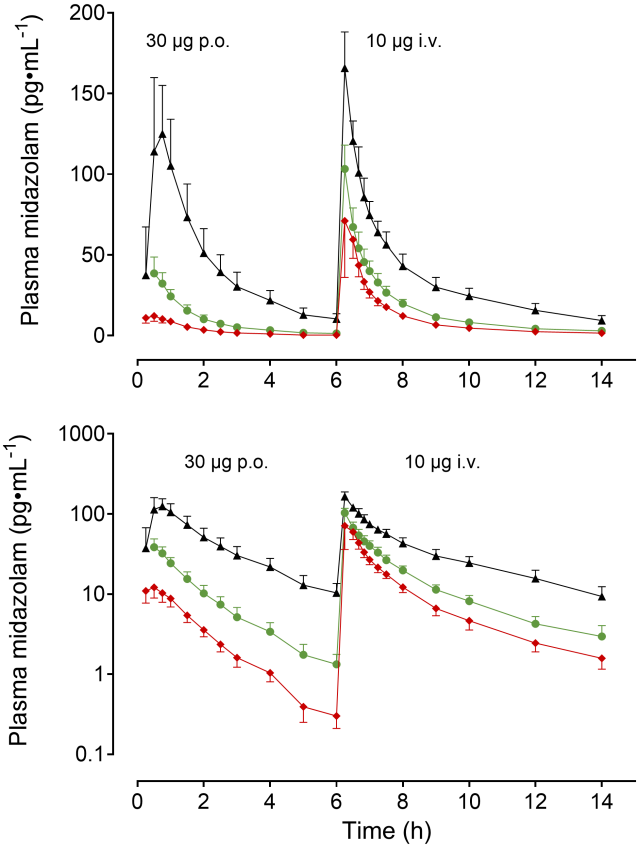

FIGURE 2 Geometric mean (95% confidence interval) midazolam plasma concentrations (upper panel: Linear concentrations; lower panel: Log concentrations) after 30 µg oral midazolam and 10 µg intravenous midazolam (administered 6 h after oral midazolam) at baseline (black triangles), during 1800 mg St. John's wort (green dots), and during 1800 mg St. John's wort plus 600 mg rifampin (red diamonds) in 11 healthy volunteers.

The contribution of extrahepatic metabolism to midazolam CL was estimated by subtracting CL_{sys} from eCL_{met}. In the absence of inducers, the estimated intestinal contribution to total midazolam CL (eCL_{met}) was 29.8%, increased to 73.5% with 1800 mg SJW, and reached 88.0% with the combined rifampin and SJW treatment.

Midazolam LSS

To evaluate the validity of the LSS in a state of CYP3A induction, we analyzed the relationship between $AUC_{2\text{-}4}$ and AUC_{∞} during the treatment with 1800 mg SJW and 1800 mg SJW plus 600 mg rifampin using a log–log regression analysis. A slope of 1.11 (95% CI: 1.08–1.21) ($R^2 = 0.975$) confirms the applicability of the abbreviated AUC methodology (Figure 3).

Using LSS data, the effect of ascending doses of SJW on total CYP3A activity (CL_{met} of midazolam) was evaluated. All SJW doses reduced midazolam exposure and increased its partial metabolic CL (Table 2, individual changes are shown in Figure S1). The addition of rifampin to the highest SJW dose further increased midazolam CL 3.11-fold (Figure 4; Table 2). Exploratory nonlinear regression analysis of the three different SJW doses and the individual increase of eCL_{met} suggests a dose close to the highest dose used required for half-maximum induction ($ED_{50} = 1720$ mg) and a maximum possible induction of 1039% (=11.4-fold increase in eCL_{met}; Figure S1). Analysis of the individual SJW dose and eCL_{met} increase showed convergence in seven out of 11 study participants (Figures S2 and S3). The single participant carrying the CYP3A5*1/*3 genotype had eCL_{met} values very similar to the population average.

Adverse events

Of 12 participants enrolled, one dropped out due to allodynia and erythrodermia,[25] two had no adverse events, and 11 had 1–5 adverse events (hypophosphatemia ($n = 6$), paresthesia (4), headache (2), nausea (2), vomiting (1), myalgia (1), hyperpigmentation (1), drop in hemoglobin (2), and pruritus (1)). All adverse events were mild-to-moderate in severity and transient. No adverse event was serious.

DISCUSSION

The data acquired in this clinical pharmacokinetic experiment in healthy volunteers provide insights into three areas: First, we have addressed important methodological aspects. It has been shown earlier[22] that the midazolam AUC obtained with an LSS after oral administration closely correlates with an extended pharmacokinetic profile. Our findings now (i) confirmed that this is also the case after administration of a microdose in the absence of a perpetrator. Our trial further showed (ii) that also during profound enzyme induction of the key metabolizing enzyme (CYP3A4), $AUC_{2\text{-}4}$ correlates well with AUC_{∞}. In this study, each dose of the paradigm inducer SJW was administered for 14 days, which has been shown to be certainly long enough to reach an induction steady state.[26] After induction of CYP3A activity with SJW administered in ascending doses, the AUC ratios correspond well with values reported in earlier trials that used the same SJW brand.[26,27] As an example, in our trial, eCL_{met} increased 3.86 times during daily administration of 900 mg SJW whereas the fold increase with a regular midazolam dose was 4.22.[26] We further evaluated (iii) how well the semi-simultaneous method first reported by Lee and coworkers,[19] which allows us to estimate CL_{sys}, CL/F, and

TABLE 1 Midazolam pharmacokinetic parameters after 30 µg oral midazolam and 10 µg intravenous midazolam (6 h after oral midazolam) at baseline, at 1800 mg St. John's wort, and 1800 mg St. John's wort plus 600 mg rifampin in 11 healthy volunteers.

	Baseline	SJW 1800		SJW 1800 + RIFAMPIN 600	
	GM (95% CI)	GM (95% CI)	GMR (90% CI)	GM (95% CI)	GMR (90% CI)
30 µg midazolam orally					
C_{max} (pg mL^{-1})	138.6 (109–177)	41.7* (31.7–54.9)	0.3008 (0.2448–0.3696)	14.0*,# (10.9–17.9)	0.1006 (0.0846–0.1196)
AUC_{0-6} (h pg mL^{-1})	257.6 (205–324)	60.7* (48.7–75.5)	0.2354 (0.1980–0.2798)	20.4*,# (16.3–25.4)	0.0790 (0.0689–0.0905)
AUC_{∞} (h pg mL^{-1})	303.1 (237.0–387.7)	65.63* (52.55–81.97)	0.2165 (0.1814–0.2584)	21.45# (17.08–26.93)	0.0707 (0.0614–0.0815)
CL/F (mL min^{-1})	1649 (1290–2109)	7619* (6100–9515)	4.620 (3.871–5.514)	23,312*,# (18,564–29,275)	14.14 (12.28–16.28)
V_z/F (L)	422 (351–507)	1612* (1195–2173)	3.819 (3.111–4.688)	4767*,# (3848–5905)	11.30 (9.433–13.53)
F (%)	35.1 (30.0–41.2)	15.6* (12.7–19.2)	0.4437 (0.3735–0.5271)	7.28*,# (5.70–9.29)	0.2070 (0.1704–0.2516)
10 µg midazolam i.v.					
C_{max} (pg mL^{-1})	155.3 (137–177)	101.9* (89.2–117)	0.6562 (0.6013–0.7161)	91.3* (72.6–115)	0.5880 (0.5082–0.6804)
AUC_{∞} (h pg mL^{-1})	334 (283–394)	145* (128–166)	0.4348 (0.3839–0.4926)	99.4*,# (83.6–118)	0.2974 (0.2569–0.3443)
Corr AUC_{∞} (h pg mL^{-1})	287 (249–332)	140* (124–159)	0.4880 (0.4336–0.5492)	98.2*,# (82.6–117)	0.3417 (0.2956–0.3949)
$t_{1/2}$ (h)	2.97 (2.61–3.37)	2.45+ (2.03–2.95)	0.8248 (0.7276–0.9350)	2.35* (2.10–2.62)	0.7908 (0.7093–0.8816)
CL_{sys} (mL min^{-1})	580 (503–669)	1188* (1046–1348)	2.049 (1.821–2.305)	1697# (1426–2019)	2.927 (2.533–3.382)
V_z (L)	148 (132–167)	251* (214–296)	1.694 (1.533–1.872)	347*,# (297–405)	2.340 (2.036–2.688)

Abbreviations: AUC_{0-6}, area under the concentration–time curve from zero to 6 h; AUC_{∞}, area under the concentration–time curve extrapolated to infinity; Corr AUC_{∞}, AUC after intravenous administration corrected for the AUC contribution from the preceding oral administration; CI, confidence interval; CL_{sys}, systemic clearance; CL/F, apparent oral clearance; C_{max}, peak plasma concentration; F, absolute bioavailability; GM, geometric mean; GMR, geometric mean ratio; SJW, St. John's Wort; $t_{1/2}$, terminal elimination half-life; V_z, volume of distribution; V_z/F, apparent volume of distribution.

*$p < 0.01$ versus baseline; +$p < 0.05$ versus baseline; #$p < 0.01$ versus SJW 1800.

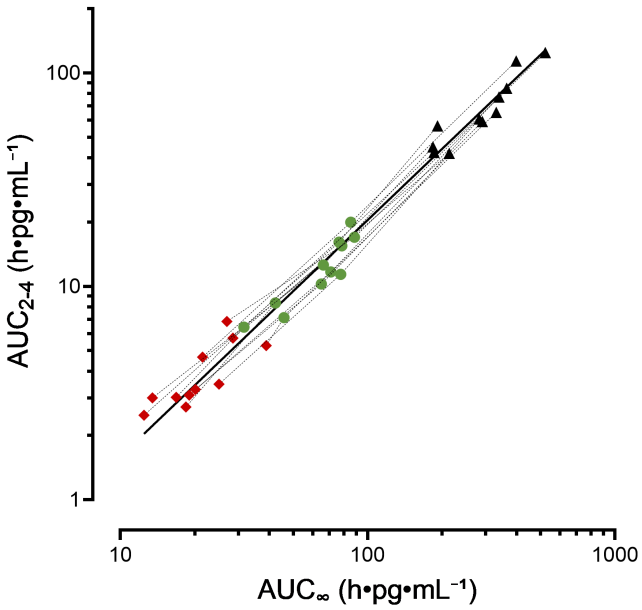

FIGURE 3 Relationship between AUC_{2-4} and AUC_{∞} of oral midazolam at baseline (black triangles), during treatment with 1800 mg SJW (green dots), during combined administration of 1800 mg SJW plus 600 mg rifampin (red diamonds) analyzed using a log–log linear regression analysis ($R^2 = 0.9753$).

absolute F, works with midazolam microdoses. The results were comparable with the data reported by others[19,28] and suggest that microdoses can also be used in this way.

Second, in this experiment, we provide insights into the dose dependence of SJW on PXR-mediated CYP3A induction. It has previously been shown that induction of different SJW formulations correlates with their content in hyperforin but not hypericin,[29,30] and our trial revealed that subtherapeutic SJW doses (300 mg q.d.) approximately doubled midazolam eCL_{met} and higher doses further but less than linearly increased eCL_{met} suggesting that a plateau will be reached. This is in line with observations with other PXR activators such as rifapentine[31] or trials with rifampin that have shown that autoinduction is saturable[32] and maximum induction is already reached at daily doses of 300–600 mg.[33]

The trial clearly confirmed that also induction by SJW is dose-dependent and estimates that the maximum increase in midazolam metabolism is approximately elevenfold, but requires doses outside the approved range and beyond good tolerance. While the induction by rifampin exceeded this estimated maximum achievable by SJW (11.4-fold increase) by ~50% (17.5-fold increase of eCL_{met} from baseline) and occurred in all participants (Figure 4), this result should be interpreted with caution as it is based on extrapolated values from only three observations per participant. In vitro, maximum effects of hyperforin were similar (PXR activation[34]), higher (CYP3A4 promoter activation[35]), or lower than the effects of rifampin (gene expression,[36] CY3A4 RNA expression[37]). In contrast, in vivo, the effect on

TABLE 2 Geometric mean (95% confidence interval) of $AUC_{2\text{-}4}$ and partial metabolic clearance of midazolam after oral administration of 30 µg oral midazolam at baseline, at 300, 900, and 1800 mg St. John's wort, and at 1800 mg St. John's wort plus 600 mg rifampin in 11 healthy volunteers.

Treatment	Duration (days)	$AUC_{2\text{-}4}$ (h pg mL^{-1})	eCL_{met} (mL min^{-1})	GMR (90% CI) of eCL_{met}
Baseline	–	65.7 (51.2–84.3)	843 (657–1081)	–
300 mg/day St. John's wort	14	33.6* (26.3–42.8)	1649* (1290–2104)	1.957 (1.661–2.305)
900 mg/day St. John's wort	14	17.0*,# (13.9–20.9)	3257*,# (2653–3999)	3.864 (3.393–4.400)
1800 mg/day St. John's wort	14	11.7*,# (9.14–15.0)	4739*,# (3705–6061)	5.621 (4.815–6.562)
1800 mg/day St. John's wort + 600 mg/day rifampin	7	3.76*,# (3.00–4.71)	14,752*,# (11,766–18,495)	17.50 (15.41–19.87)

Note: Test versus baseline: *$p < 0.001$; Test versus next lower St. John's Wort dose: #$p < 0.001$.

Abbreviations: $AUC_{2\text{-}4}$, area under the concentration–time curve from 2 to 4 h; CI, confidence interval; eCL_{met}, estimated midazolam partial metabolic clearance; GMR, geometric mean ratio.

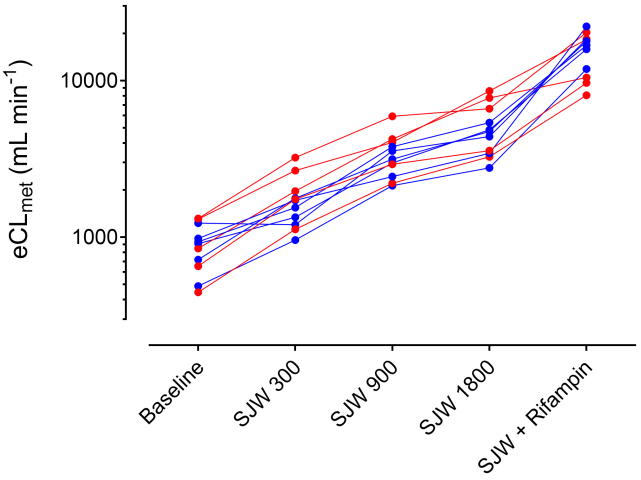

FIGURE 4 Individual estimated metabolic midazolam clearance (eCL_{met}) in relation to different induction conditions (baseline; SJW 300 = St. John's wort 300 mg for 14 days; SJW 900 = 900 mg for 14 days; SJW 1800 = 1800 mg for 14 days; SJW + Rifampin = 1800 mg SJW together with 600 mg rifampin for 7 days), calculated by means of the limited-sampling strategy in 11 healthy volunteers (female = red symbol; male = blue symbol).

the exposure of paradigm CYP3A4 substrates (ciclosporin,[38] nifedipine,[39] tacrolimus[40]) was substantially more pronounced with rifampin than with SJW (ciclosporin,[41] nifedipine,[42] tacrolimus[43]), thus well matching the results of this trial. The reason for this apparent discrepancy is not known; considering that the molecular weight of hyperforin is 536.8 g/Mol, the molar concentration of hyperforin was 83.8 µM in the 200 mL drinking water, which is ~9-fold lower than the corresponding molar concentrations of rifampin (729 µM, molecular weight: 822.9 g/Mol) and for both compounds exceeds EC_{50} of their binding to PXR by orders of magnitude. It appears therefore unlikely that the numerically higher rifampin concentrations will displace relevant amounts of the more tightly bound hyperforin from PXR. Much more likely is that free rifampin concentrations in blood and tissue[44] exceed free hyperforin concentrations by orders of magnitude.

Estimation of the intestinal contribution of enzyme induction to overall metabolism revealed that the induction was predominantly due to an increase in intestinal CL. Our calculation may even slightly underestimate the true contribution of intestinal metabolism, as a small proportion of circulating midazolam is also cleared extrahepatically.[45]

Interaction trials thoroughly evaluating the combined effect of two enzyme-inducing agents are rare. A trial with therapeutic doses of bosentan in healthy volunteers revealed that the bosentan-induced (auto)induction of CYP3A4 could be further increased by 47% by adding the same dose (900 mg/day) and brand of SJW as administered in this trial.[46] This was also observed with rifampin, which also increased the (auto)induction of therapeutic bosentan doses,[47] but not beyond a range observed with supratherapeutic bosentan doses alone.[48] In another trial in healthy volunteers, 900 mg SJW of another brand had no effect on carbamazepine-induced (auto)induction[49] but reduced exposure to single carbamazepine doses by 21% in the absence of autoinduction.[50]

When comparing the dose-normalized data of an earlier rifampin–midazolam interaction trial with our microdose trial, similar results were obtained.[51] Thus, these results suggest that the combination of the two PXR activators did not result in a stronger induction than rifampin alone and that the maximal induction differs between the two compounds. In addition to the possibility that this is a consequence of different binding properties at the corresponding PXR pocket,[13,17] it could also be caused by modifications further downstream of the signaling cascade that modify PXR translocation from the cytoplasm into the nucleus[52] or by post-translational changes of the hinge region of PXR[53] that alter the interaction with the DNA.

The findings of this trial have several implications. First, this study clearly shows that the extent of CYP3A4 induction induced by SJW does not only depend on the specific brand and its hyperforin content[29] but also on the administered dose. Although this could be anticipated from in vitro studies,[15] this has not been thoroughly investigated before and only assessed with two doses of hypericum powder.[54] Our trial also showed that the maximum CYP3A4 induction is not reached with the maximum recommended dose of this brand (900 mg/day), suggesting that administration of brands with higher hyperforin content (e.g., Neuroplant®, Dr. Willmar Schwabe GmbH, Karlsruhe, Germany, hyperforin content 12.43 mg/300 mg[21]) will cause even stronger induction and more pronounced drug–drug interactions. The results of interaction studies with the highest approved dose of a certain SJW brand are therefore not readily transferable to other phytotherapeutic generics because their composition can differ considerably. This also shows that it is important to report the specific phytotherapeutic brand administered together with its content of relevant constituents.

Second, this trial also suggests that compounds that induce CYP via the same mechanism (PXR) may differ significantly in the magnitude of induction they exert and that SJW is a weaker inducer than rifampin despite the higher affinity of hyperforin to the ligand-binding pocket. Whether this is due to fundamental differences in the activation of the PXR cascade needs to be clarified in vitro. Together with the results of our trial, these data indicate that SJW is less potent inducer than rifampin, carbamazepine, and bosentan, and suggest that when SJW is combined with these inducers, the effects of the stronger inducers will determine the magnitude of induction.

Finally, the pharmacokinetic changes observed with microdoses of midazolam correspond well with the changes observed when regular doses were administered with SJW, suggesting that microdoses reflect the interaction well even in an induced state. In addition, the close correlation with abbreviated sampling protocols suggests that the LSS can be used also for this purpose.

The limitations of this trial are (i) the short duration of rifampin treatment, which was, however, considered long enough to achieve maximum induction,[55] (ii) the absence of a control group receiving rifampin alone, and (iii) that no information on the effect of rifampin on the pharmacokinetics and thus exposure of hyperforin was obtained, which is a substrate of the CYP2C and CYP3A enzyme families.[56] Finally, (iv) a change in the volume of distribution of midazolam after i.v. administration was noted in our trial, which has not been observed previously[57] and is most likely due to a decrease in serum albumin levels from 46.0 g/L (95% CI: 44.6–47.5) at screening to 41.4 g/L (39.5–43.3; $p < 0.0001$) at the end of the study, which may significantly increase the volume of distribution of midazolam[58] and is likely due to the withdrawal of slightly more than 400 mL of blood during the trial.

In conclusion, we have shown in this trial in healthy volunteers that the inducing effect of SJW is dose-dependent and can be substantially enhanced by the addition of rifampin, suggesting that the two inducers may differ mechanistically. At the same time, we have shown that the pharmacokinetics of midazolam microdoses also reflect the pharmacokinetic changes during the administration of CYP3A inducers and compare well with the known changes reported after the administration of regular doses of midazolam.

AUTHOR CONTRIBUTIONS

N.H., G.M., and W.E.H. wrote the manuscript. N.H., G.M., and W.E.H. designed the research. N.H., A.S.F., A.B., and G.M. performed the research. G.M., J.B., and N.H. analyzed the data.

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