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PHARMACOKINETICS AND DISPOSITION

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Rifampicin is only a weak inducer of CYP1A2-mediated presystemic and systemic metabolism: studies with tizanidine and caffeine

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Abstract *Objective:* Rifampicin greatly reduces the plasma concentrations of many drugs. Our aim was to characterise the inducibility of cytochrome P450 (CYP) 1A2 by rifampicin, using tizanidine and caffeine as probe drugs for presystemic and systemic CYP1A2-mediated metabolism. *Methods:* In a randomised, 2-phase cross-over study, ten healthy volunteers were given a 5-day pretreatment with 600 mg rifampicin or placebo once daily. On day 6, a single 4-mg dose of tizanidine was administered orally. Plasma and urine concentrations of parent tizanidine and several of its metabolites (M-3, M-4, M-5, M-9, M-10) and pharmacodynamic variables were measured up to 24 h. A caffeine test was performed in both phases. *Results:* Rifampicin moderately reduced the peak plasma concentration (by 51%; P=0.002) and area under the plasma concentration-time curve [AUC(0–∞)] (by 54%; P=0.009) of parent tizanidine, and had no effect on its half-life. The tizanidine/M-3 and tizanidine/M-4 AUC(0–∞) ratios were slightly (by 30%; P=0.014; and by 38%; P=0.007) decreased by rifampicin. Also, the excretion of metabolites M-3, M-4 and M-5 into urine was reduced (P<0.005), but that of M-10 was increased (P=0.008) by rifampicin. Rifampicin reduced the tizanidine/M-10 ratio (by 55%; P=0.047) but had no significant effect on the other tizanidine/metabolite ratios in urine. The caffeine/paraxanthine ratio was reduced by 23% (P=0.081) by rifampicin. The effect of rifampicin on the caffeine/paraxanthine ratio correlated significantly with the effect of rifampicin on, for example, the AUC(0–∞) of tizanidine and the tizanidine/M-3 AUC(0–∞) ratio. The pharmacodynamic effects of tizanidine were reduced by rifampicin. *Conclusions:* Rifampicin moderately decreases the plasma concentrations of tizanidine. The strong inducing effects of rifampicin on other CYP enzymes, e.g. CYP3A4, may have contributed to the findings, and the inducibility of CYP1A2-mediated presystemic (tizanidine) and systemic (tizanidine, caffeine) metabolism by rifampicin is weak at the most. Compared to CYP3A4 substrate drugs, substrates of CYP1A2 are much less susceptible to drug interactions caused by enzyme inducers of the rifampicin type.

Keywords Rifampicin · Tizanidine · CYP1A2 · Enzyme induction · Interaction

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The rifamycin antibiotic rifampicin is a potent inducer of many drug-metabolising enzymes and transport proteins [1–3]. In particular, it can greatly (more than 10-fold) reduce the plasma concentrations and effects of drugs with significant presystemic metabolism, such as the cytochrome P450 3A4 (CYP3A4) substrates midazolam [4], triazolam [5], buspirone [6] and simvastatin [7]. Rifampicin also induces the expression of some drug transporters, e.g. the efflux transporters P-glycoprotein [8] and canalicular multispecific organic anion transporter (cMOAT, or MRP2) [9].

There have been only a few studies on the possible inducing effect of rifampicin on CYP1A2 in vivo. Rifampicin has been shown to modestly reduce the area under the concentration-time curve (AUC) of orally administered theophylline (by 18–45%) [3, 10, 11] and caffeine (by about 40%) [12, 13], and that of intravenously administered ropivacaine (by 52% in nonsmokers and by 38% in smokers) [14]. However, theophylline, caffeine and ropivacaine are metabolised to some extent also by other enzymes, including CYP2C9, CYP2E1 and CYP3A4, and theophylline is a substrate for organic anion transporter 2 [15–25]. CYP3A4 can be especially strongly induced by rifampicin; since the basic mechanisms of regulation and induction of CYP1A2 differ from those of CYP3A4 [26, 27], the inducibility of CYP1A2 by rifampicin in humans still remains uncertain. Moreover, the studied CYP1A2 substrates are low clearance drugs with no significant

presystemic metabolism, and the effect of rifampicin on the pharmacokinetics of orally administered probe drugs, which have a significant CYP1A2 mediated presystemic metabolism, is not known.

Caffeine has been used widely as an in vivo probe of hepatic CYP1A2 activity [28–31]. Different caffeine-based indices, e.g. the ratio of caffeine and paraxanthine in plasma, reflect mainly systemic elimination by CYP1A2 [32]. Tizanidine, a centrally acting skeletal muscle relaxant, is eliminated from the body principally by CYP1A2-mediated biotransformation into several metabolites (Fig. 1) [33–35]. However, tizanidine differs pharmacokinetically from caffeine by its extensive presystemic metabolism and shorter elimination half-life [32, 33, 35]. Fluvoxamine and ciprofloxacin, inhibitors of CYP1A2 [36, 37], strongly increase the plasma concentrations and effects of tizanidine in vivo [35, 38]. Accordingly, tizanidine is a very sensitive CYP1A2 probe drug, which can be used to measure presystemic CYP1A2 mediated metabolism in humans.

In order to study the inducibility of presystemic and systemic CYP1A2-mediated metabolism by rifampicin in humans, we have investigated the effect of rifampicin on the pharmacokinetics and metabolite profile of tizanidine, and whether these effects parallel changes in the caffeine test. To this end, a carefully controlled rifampicin-tizanidine interaction study was conducted in healthy subjects.

Methods

Subjects

Ten healthy volunteers (six men and four women; age range 19–25 years; weight range 50–85 kg) participated in the study after giving written informed consent (Table 1). The volunteers were ascertained to be healthy by medical history, physical examination and routine laboratory tests before entering the study. For safety reasons, subjects with a systolic blood pressure lower than 110 mmHg were excluded from the study. None of the subjects were tobacco smokers and none used oral contraceptives or other continuous medication. The sample size was chosen so that a possible clinically significant pharmacokinetic drug interaction can be verified statistically without the use of an unnecessarily large group of healthy subjects. The number of subjects was estimated to be sufficient to detect a 30% change in the AUC(0–∞) of tizanidine with a power of 80% (alpha-level 5%).

Study design

The study protocol was approved by the Ethics Committee for Studies in Healthy Subjects of the Hospital District of Helsinki and Uusimaa, and the Finnish National Agency for Medicines. A randomised, 2-phase crossover study with a washout period of 4 weeks was carried out. The volunteers received 600 mg rifampicin (one Rimapen 600 mg tablet; Orion Pharma, Espoo, Finland), or placebo once daily at 1600 hours for 5 days. On day 6, after an overnight fast, a single oral dose of 4 mg tizanidine (one Sirdalud 4 mg tablet; Novartis Pharma, Wehr, Germany) was administered with 150 ml water at 0900 hours. A standard meal was served 4 and 7 hours after tizanidine administration. Drinking of grapefruit juice and tobacco smoking were not allowed for 1 week before each study day. Alcohol and drinks containing caffeine were not permitted on the study days.

An oral caffeine test was performed on the 5th day of the pretreatment during both phases [28–31]. The subjects ingested 100 mg caffeine (one Cofi-Tabs 100 mg tablet; Vitabalans, Hämeenlinna, Finland) at 0900 hours, after

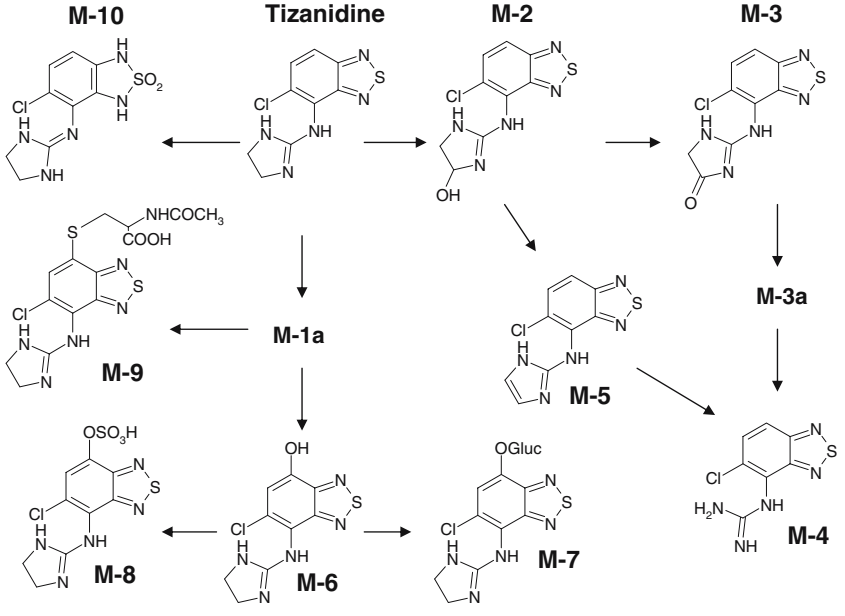

Fig. 1 Metabolic pathways of tizanidine according to Koch et al. 1989 [33]

Table 1 Characteristics of the subjects

Subject No.	Sex	Age (years)	Weight (kg)	Caffeine / paraxanthine ratio	
				Placebo phase (control)	Rifampicin phase
1	Female	20	50	1.62	1.20
2	Female	20	55	2.42	1.05
3	Male	24	81	1.03	0.79
4	Male	19	76	0.89	0.82
5	Male	21	68	0.86	0.97
6	Female	20	71	1.03	0.68
7	Male	23	83	1.75	1.16
8	Male	23	75	0.84	0.76
9	Female	25	53	1.46	1.63
10	Male	24	85	0.95	0.83
Mean±SD		21±2	71±11	1.28±0.52	0.99±0.29*

*P=0.081, versus control

having abstained from intake of caffeine for at least 18 h, and a blood sample for analysis of plasma caffeine and paraxanthine (1,7-dimethylxanthine) was taken from each subject 6 h after caffeine intake.

The subjects were under direct, close medical supervision for 12 h after administration of tizanidine. Fluids for intravenous infusion were available for immediate use, but were not needed.

Sampling

On the days of tizanidine administration, a forearm vein of each subject was cannulated with a plastic cannula and kept patent with an obturator. Timed blood samples were drawn before the administration of tizanidine and at 20, 40, 60 and 90 min and 2, 3, 4, 5, 7, 9, 12 and 24 h later. Blood samples (10 ml each) were taken into ethylenediaminetetra-acetic acid containing tubes. Plasma was separated within 30 min. Urine was collected cumulatively in two fractions: 0–12 and 12–24 h. Urine aliquots and plasma were stored at −70°C until analysis.

Drug concentrations in plasma and urine

Plasma and urine tizanidine and metabolite (M) concentrations were quantified by use of an API 2000 liquid chromatography-tandem mass spectrometry system (MDS Sciex, Toronto, Ontario, Canada). Chromatography was performed on an XTerra RP C18 column (3.9×100 mm; Waters, Milford, Mass) using gradient elution. The mobile phase consisted of 10 mM ammonium acetate (pH 9.5, adjusted with 25% ammonia solution) and acetonitrile. The mass spectrometer was operated in the atmospheric pressure chemical ionisation (APCI) mode with positive ion detection. The ion transitions monitored were mass-to-charge ratio (m/z) 254 to m/z 44 for tizanidine, m/z 268 to m/z 211 for M-3, m/z 228 to m/z 211 for M-4, m/z 252 to m/z 216 for M-5, m/z 415 to m/z 286 for M-9, m/z 288 to m/z 188 for M-10, and m/z 230 to m/z 44 for the internal standard, clonidine. These transitions represent the product ion of the $[M+H]^+$ ion. The limit of quantification for tizanidine was 0.05 ng/ml and the day-to-day coefficient of variation (CV) was 17.5% at 0.1 ng/ml, 7.3% at 1.2 ng/ml and 13.7% at 12.8 ng/ml (n=5). A signal to noise ratio of 10:1 was used as the limit of detection for tizanidine metabolites, and their concentrations are given in arbitrary units relative to the ratio of the peak height of the metabolite to the peak height of the internal standard. The detector response for each metabolite was confirmed to be linear over the relevant concentration range by means of sample dilution. Rifampicin did not interfere with the determinations.

Plasma caffeine and paraxanthine concentrations were determined by HPLC, with β-OH-ethyltheophylline used as the internal standard [39, 40]. The day-to-day CV of caffeine and paraxanthine was less than 6% at relevant concentrations.

Pharmacokinetics

The pharmacokinetics of tizanidine, and metabolites 3 and 4, were characterised by peak concentration in plasma (C_{max}), time to C_{max} (t_{max}), area under the plasma concentration-time curve from 0 to infinity [AUC(0–∞)], and elimination half-life ($t_{½}$) using non-compartmental methods as described earlier [35, 38]. In addition, the amount of tizanidine and its metabolites excreted into urine within 24 h (Ae) were calculated. The renal clearance (Cl_{renal}) of tizanidine was calculated as Cl_{renal} = Ae/AUC (0–24).

Pharmacodynamics

Systolic and diastolic blood pressures, heart rate, subjective drowsiness, subjective overall drug effect and the digit symbol substitution test (DSST) were assessed before administration of tizanidine and immediately after each

blood sampling, as described earlier [35, 38]. For each pharmacodynamic variable, the area under the effect versus time curve from 0 to 12 h [AUC(0–12 h)] was calculated by use of the trapezoidal rule. In addition, the maximum responses in each pharmacodynamic variable were recorded.

Statistical analysis

Results are expressed as mean values±standard deviation (SD) in the tables and text and, for clarity, as mean values± standard error of the mean (SEM) in the figures. The pharmacokinetic and pharmacodynamic variables and the caffeine/paraxanthine ratio during the placebo and rifampicin phase were compared by repeated-measures ANOVA with treatment sequence as a factor or, in the case of t_{max} with the Wilcoxon signed-rank test. For all variables except t_{max}, 95% confidence intervals (CI) were calculated on the mean differences between the placebo and rifampicin phase. The Pearson correlation coefficient was used to investigate possible relationships of the pharmacokinetic variables of tizanidine, the tizanidine/metabolite AUC (0–∞) ratios and the tizanidine/metabolite urinary excretion (Ae) ratios with the caffeine/paraxanthine ratio during the placebo phase and the possible relationships between their changes caused by rifampicin. All the data were analysed with the statistical program Systat for Windows, version 6.0.1. (SPSS, Chicago, Ill). Differences were considered statistically significant at $P<0.05$.

Results

Plasma tizanidine

Rifampicin moderately decreased the mean C_{max} of tizanidine (by 51%; $P=0.002$) and its AUC(0–∞) (by 54%; $P=0.009$) (Fig. 2, Table 2). There was little variation in the extent of the interaction, but in one of the ten subjects, the C_{max} and AUC(0–∞) were not decreased at all (Fig. 3a,c). Furthermore, the mean $t_{½}$ and t_{max} of tizanidine were unaffected by rifampicin (Figs. 2, 3b, Table 2).

Tizanidine metabolites M-3 and M-4 in plasma

Rifampicin slightly reduced the C_{max} of M-3 (17%; $P=0.044$) and M-4 (10%; $P=0.050$), and shortened the t_{max} of M-4 (from 2.5 to 1.0 h; $P=0.014$) (Fig. 4, Table 2). The $t_{½}$ for M-3 was shortened from 4.1 to 3.6 h ($P=0.031$). Rifampicin also decreased the AUC(0–∞) of both M-3 (33%; $P=0.003$) and M-4 (23%; $P<0.001$), as well as the tizanidine/M-3 AUC(0–∞) ratio (30%; $P=0.014$), and the tizanidine/M-4 AUC(0–∞) ratio (38%; $P = 0.007$). The greatest decrease in plasma tizanidine/metabolite concentration ratio was observed during the absorption phase, about 20–120 min after tizanidine administration (Fig. 4).

Excretion of tizanidine and its metabolites into urine

Rifampicin did not change significantly the Ae ($P=0.13$) or Cl_{renal} of the parent tizanidine ($P=0.14$) (Fig. 5). The Ae of M-3, M-4 and M-5 was decreased ($P<0.005$), but that of M-10 was increased by rifampicin ($P=0.008$). Rifampicin decreased the tizanidine/M-10 urinary excretion ratio by

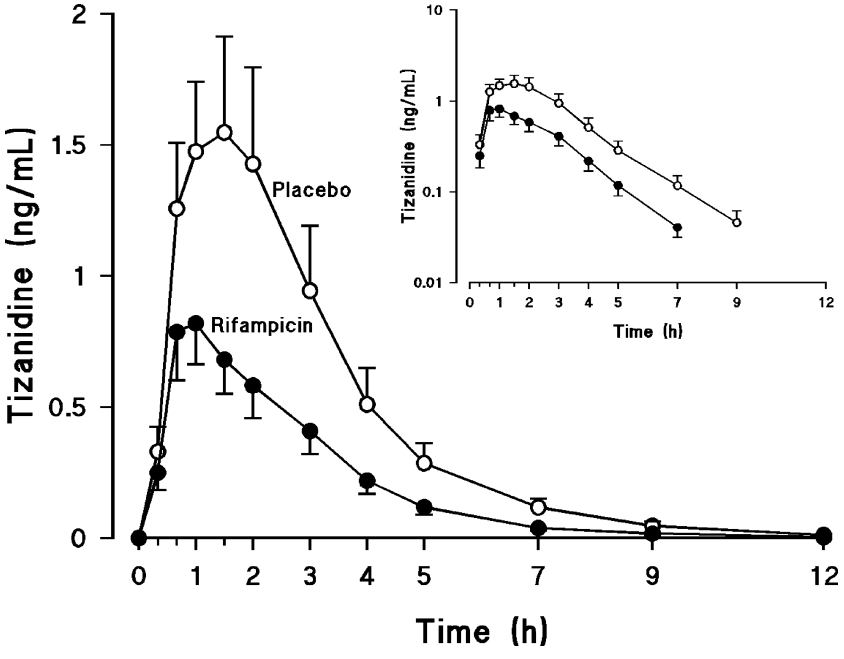

Fig. 2 Plasma concentrations (mean±SEM) of tizanidine in ten healthy volunteers after a single oral dose of 4 mg tizanidine, after treatment with placebo (*open circles*) or 600 mg rifampicin (*solid circles*) daily for 5 days. *Inset* Same data on a semi-logarithmic scale

Table 2 Pharmacokinetic variables of 4 mg tizanidine and its metabolites M-3 and M-4 in ten healthy subjects after pretreatment with placebo or 600 mg rifampicin once daily for 5 days

Variable	Placebo phase (control)	Rifampicin phase	Difference between phases (95% CI)	*P* value
Tizanidine				
C_{max} (ng/ml)	1.77±1.11	0.88±0.59	−0.90 [(−1.38) – (−0.43)]	0.002
% of control and range	100	49 (26–123)		
t_{max} (min)	60 (40–90)	60 (40–60)		0.20
$t_{½}$ (h)	1.4±0.2	1.4±0.2	0 [(−0.1) – 0.1]	0.98
% of control and range	100	100 (86–123)		
AUC(0–∞) (ng·h/ml)	5.16±3.75	2.37±1.55	−2.78 [(−4.66) – (−0.91)]	0.009
% of control and range	100	46 (26–121)		
Cl_{renal} (L/h)	3.33±1.14	4.15±1.72	0.82 [(−0.32) – 1.96]	0.14
% of control and range	100	125 (41–245)		
M-3				
C_{max} (U/ml)	1.12±0.72	0.94±0.62	−0.18 [(−0.36) – (−0.01)]	0.044
% of control and range	100	83 (63–126)		
t_{max} (min)	120 (40–180)	75 (40–180)		0.24
$t_{½}$ (h)	4.1±0.8	3.6±0.9	−0.5 [(−1.0) – (−0.1)]	0.031
% of control and range	100	87 (63–120)		
AUC(0–∞) (U·h/ml)	6.22±3.72	4.19±2.70	−2.03 [(−3.13) – (−0.93)]	0.003
% of control and range	100	67 (50–99)		
AUC(0–∞) ratio (ng/U); (tizanidine/M-3)	0.84±0.52	0.59±0.34	−0.25 [(−0.44) – (−0.07)]	0.014
% of control and range	100	70 (48–123)		
M-4				
C_{max} (U/ml)	1.31±0.24	1.18±0.22	−0.13 [(−0.25) – 0]	0.050
% of control and range	100	90 (78–119)		
t_{max} (min)	150 (40–240)	60 (40–120)		0.014
$t_{½}$ (h)	4.5±0.3	4.5±0.3	0 [(−0.2) – 0.3]	0.76
% of control and range	100	101 (94–113)		
AUC(0–∞) (U·h/ml)	10.48±2.77	8.14±2.34	−2.35 [(−3.10) – (−1.59)]	< 0.001
% of control and range	100	77 (64–87)		
AUC(0–∞) ratio (ng/U); (tizanidine/M-4)	0.46±0.26	0.28±0.17	−0.18 [(−0.29) – (−0.06)]	0.007
% of control and range	100	62 (34–146)		

Data are mean values ±SD or mean with 95% CI; percentage of control is given with range; t_{max} data are given as median with range.
CI Confidence interval, C_{max} peak concentration, t_{max} time to reach C_{max}, $t_{½}$ half-life, *AUC(0–∞)* area under the plasma concentration-time curve from time 0 to infinity, Cl_{renal} renal clearance

55% (*P*=0.047), but had no statistically significant effect on the urinary excretion ratios of tizanidine to the metabolites M-3, M-4, M-5 or M-9.

Pharmacodynamic variables

In the placebo phase, tizanidine reduced the systolic and diastolic blood pressures and heart rate from baseline values by −17 mmHg, −13 mmHg and −12 beats/min, respectively (Table 3). During the rifampicin phase, the corresponding reductions were −10 mmHg (*P*=0.059, versus placebo phase), −9 mmHg (*P*=0.035) and −7 beats/min (*P*=0.054), respectively. There were no significant differences between the phases in the effects of tizanidine on subjective drowsiness, overall drug effect, or DSST (Table 3).

Caffeine test

Rifampicin decreased the plasma caffeine/paraxanthine concentration ratio by 23%, but this change was statistically non-significant (*P*=0.081) (Table 1).

Correlations between tizanidine pharmacokinetics and caffeine/paraxanthine ratio and their changes by rifampicin

The AUC(0–∞), C_{max} and Ae of the parent tizanidine during the placebo phase, and their changes caused by rifampicin, correlated significantly (*P*<0.05) with the caffeine/paraxanthine ratio during the placebo phase, and with the change of the caffeine/paraxanthine ratio with rifampicin (Table 4). Furthermore, during placebo, the tizanidine/M-3 and tizanidine/M-4 AUC(0–∞) ratios, the

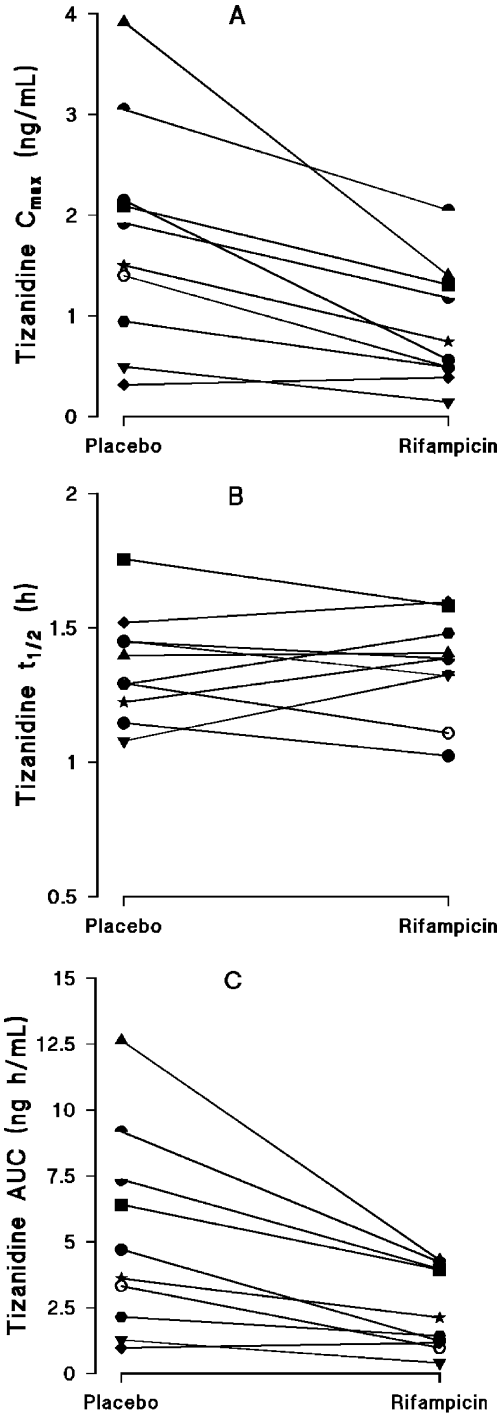

Fig. 3 Individual values for peak concentration in plasma (C_{max}) (**a**), elimination half-life ($t_{½}$) (**b**), and area under plasma concentration-time curve from time 0 to infinity [AUC(0–∞)] (**c**) of tizanidine in ten healthy volunteers after a single oral dose of 4 mg tizanidine after treatment with placebo or 600 mg rifampicin daily for 5 days

Ae of M-3 and M-5, and all tizanidine/metabolite Ae ratios correlated significantly with the caffeine/paraxanthine ratio (P<0.01). Moreover, the changes in the tizanidine/M-3 AUC(0–∞) ratio, Ae of M-5, tizanidine/M-4 Ae ratio, tizanidine/M-5 Ae ratio and tizanidine/M-10 Ae ratio correlated with the change caused by rifampicin in the caffeine/paraxanthine ratio (P<0.05).

Discussion

The results of the present study show that administration of rifampicin 600 mg daily for 5 days only weakly affects the pharmacokinetics of tizanidine, a CYP1A2 substrate with an extensive presystemic metabolism, and the caffeine test, an index of hepatic CYP1A2 activity. Rifampicin decreased the C_{max} and AUC(0–∞) of tizanidine by about 50%, and had no effect on its $t_{½}$ or renal clearance. An identical short-term pretreatment with rifampicin has decreased the C_{max} and AUC of several drugs, e.g. midazolam, triazolam, buspirone and simvastatin, which have a considerable CYP3A4-mediated presystemic metabolism, by 10- to 30-fold more than this [4–7].

Induction of several CYP enzymes, e.g. CYP3A4, and of other proteins by rifampicin is mediated by the pregnane X receptor (PXR) [27, 41, 42]. However, induction of CYP1A2 is mediated by the aryl hydrocarbon receptor (Ahr) [26, 27], and there is no direct evidence about the involvement of PXR in the regulation of CYP1A2. The in vitro effects of the PXR ligand rifampicin on CYP1A2 expression have been variable. For example, in cultured human hepatocytes, rifampicin has induced expression of CYP1A2 mRNA much less (0.5-fold reduction to 2-fold increase) than that of CYP3A4 (more than 50-fold increase), CYP2B6 (about 9-fold), CYP2C8 (about 6.5-fold), CYP2C9 (about 4-fold), or CYP3A5 (about 5-fold) [43]. In other in vitro studies, rifampicin has had either no effect [44–47] or it has increased (even several-fold) CYP1A2 expression or activity [48, 49]. In a recent study, rifampicin induced expression of CYP1A2 mRNA by 3- to 26-fold, and that of Ahr 2- to 3-fold in cultured human hepatocytes, suggesting that rifampicin can increase CYP1A2 expression by inducing Ahr [48]. Our present findings are consistent with these in vitro results, and indicate that CYP1A2 is only weakly induced by rifampicin in humans.

The oral bioavailability of tizanidine is only about 10–30% due to extensive presystemic metabolism [33, 38, 50], with CYP1A2 being crucial to its metabolism [34, 35]. Fluvoxamine and ciprofloxacin, inhibitors of CYP1A2 [36, 37], have drastically increased the AUC (33-fold and 10-fold, respectively) of tizanidine [35, 38], suggesting that the contribution of CYP1A2 to the total elimination of tizanidine would be at least 90–97%. The main metabolites of tizanidine found in human plasma are M-3, M-4 and M-10 [33]. In addition to these, significant amounts of M-5 and M-9 were excreted into urine. The enzymes responsible for the formation of tizanidine metabolites have not been fully characterised. However, preliminary in vitro results (M.J. Karjalainen et al., unpublished data) from our laboratory indicate that CYP1A2 mediates the formation of M-3, M-4 and M-5. In our present study, the AUC(0–∞) and C_{max} of the parent tizanidine, and the different tizanidine/metabolite ratios correlated significantly with

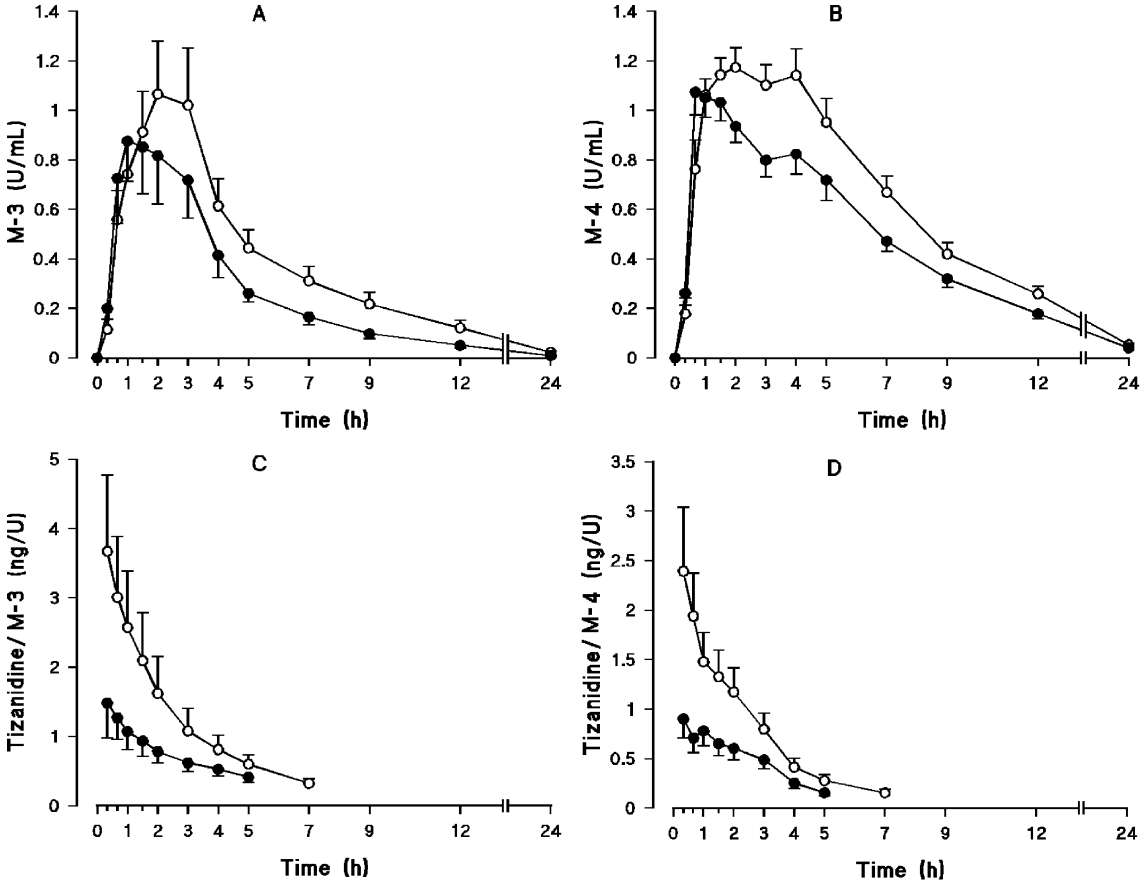

Fig. 4 Plasma concentrations (mean±SEM) of tizanidine metabolites M-3 (**a**) and M-4 (**b**), and the tizanidine/M-3 ratio (**c**) and tizanidine/M-4 ratio (**d**), in ten healthy volunteers after a 4 mg oral dose of tizanidine following pretreatment with placebo (*open circles*) or 600 mg rifampicin (*solid circles*) daily for 5 days

Table 3 Maximum change from baseline value (minimum or maximum) and incremental or decremental AUC(0–12) for systolic and diastolic blood pressure, heart rate, and psychomotor tests (subjective drowsiness, subjective drug effect and DSST), after a single oral 4-mg dose of tizanidine given after pretreatment with placebo or 600 mg rifampicin once daily for 5 days in ten healthy volunteers

Variable	Placebo phase (control)	Rifampicin phase	Difference between phases (95% CI)	*P* value
Systolic blood pressure				
Minimum (mmHg)	−17±7	−10±7	7 (0–15)	0.059
Decremental AUC(0–12) (mmHg h)	−61±63	20±51	82 (26–137)	0.010
Diastolic blood pressure				
Minimum (mmHg)	−13±4	−9±4	4 (0–8)	0.035
Decremental AUC(0–12) (mmHg h)	−79±52	−27±42	52 (12–93)	0.018
Heart rate				
Minimum (beats/min)	−12±9	−7±4	5 [(−0.1)–10]	0.054
Decremental AUC(0–12) (beats/min h)	6±113	32±73	27 [(−25)–79]	0.27
Drowsiness (VAS)				
Maximum (mm)	25±17	15±17	−10 [(−24)–3]	0.12
Incremental AUC(0–12) (mm h)	8±152	−126±291	−133 [(−340)–74]	0.18
Drug effect (VAS)				
Maximum (mm)	13±19	7±9	−6 [(−17)–4]	0.20
Incremental AUC(0–12) (mm h)	33±63	18±32	−15 [(−55)–25]	0.42
DSST				
Minimum (symbols/2 min)	−8±6	−8±5	1 [(−5)–6]	0.80
Decremental AUC(0–12) (symbols/2 min h)	−22±61	−12±61	10 [(−48)–68]	0.70

Data are mean values±SD or mean with 95% CI. *AUC(0–12)* Area under effect versus time curve from time 0 to 12 h, *VAS* visual analog scale, *DSST* digit symbol substitution test

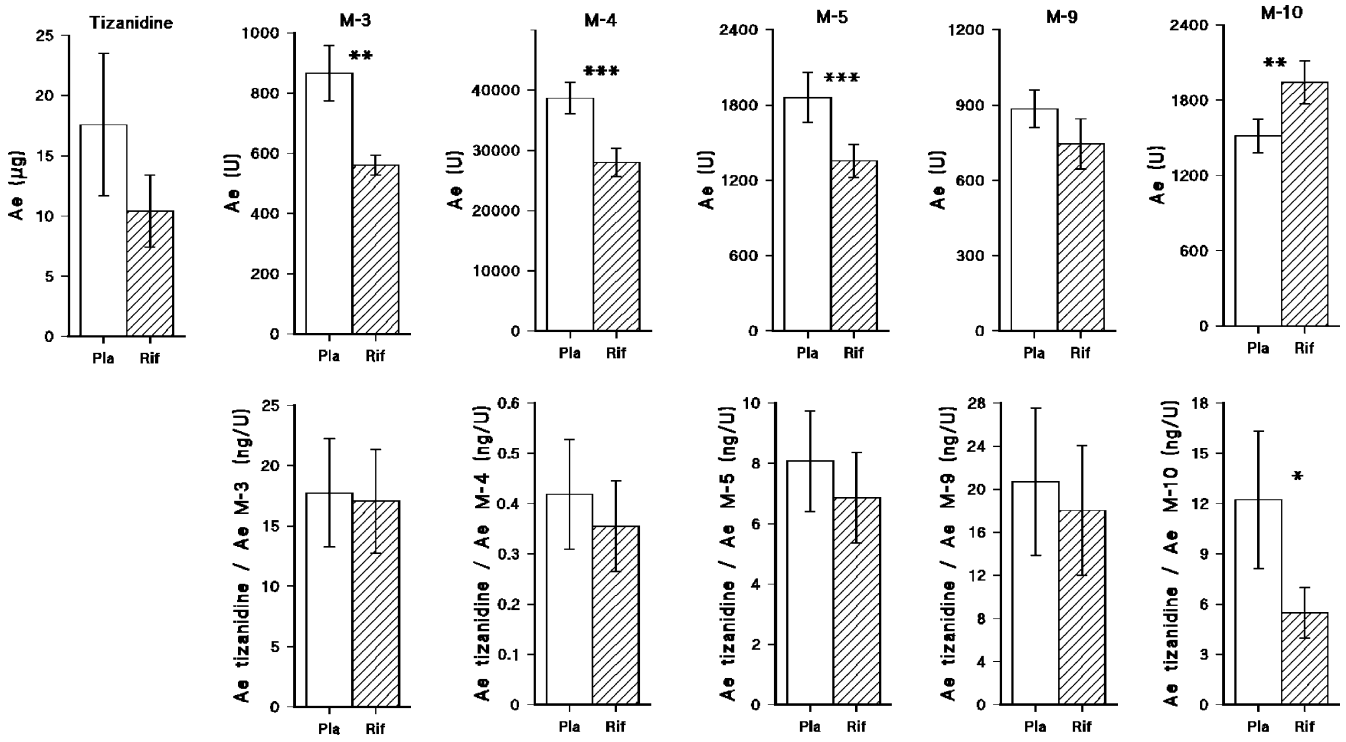

Fig. 5 Amounts excreted into urine within 24 h (Ae) (mean±SEM) of tizanidine, and its metabolites M-3, M-4, M-5, M-9 and M-10, and urine tizanidine/metabolite ratios, in ten healthy subjects after a 4 mg oral dose of tizanidine following pretreatment with placebo (*open bars*) or 600 mg of rifampicin (*hatched bars*) daily for 5 days. *P<0.05, **P<0.01, ***P<0.001

the caffeine/paraxanthine ratio during the placebo phase, supporting the role of CYP1A2 in the formation of tizanidine metabolites [51].

In the present study, rifampicin reduced the tizanidine/M-3 and tizanidine/M-4 ratios in plasma and shortened the t_{max} of M-4, consistent with induction of the formation of M-3 and M-4 by rifampicin. The rate of biotransformation of tizanidine to M-3 and M-4 was increased by rifampicin especially during the absorption phase, as seen in the ascending portions of the plasma M-3 and M-4 concentration-time curves, and the over 50% reduction in the plasma tizanidine/metabolite ratios at 20 min–2 h after tizanidine intake (Fig. 4). The effect of rifampicin on the AUC of tizanidine and the tizanidine/M-3 and tizanidine/M-4 AUC-ratios was in correlation with its effect on the caffeine/paraxanthine ratio, strongly suggesting that the increase in M-3 and M-4 formation by rifampicin was due to induction of CYP1A2. It seems that rifampicin also induced the further metabolism of M-3, M-4 and M-5, which probably explains the reduced AUC values and urinary excretions (Ae) of these metabolites and the shortened $t_{½}$ of M-3. Rifampicin had no statistically significant effect on the tizanidine/M-3, tizanidine/M-4, tizanidine/M-5 and tizanidine/M-9 Ae ratios (Fig. 5), which may be explained by variability in the renal clearance of tizanidine and its metabolites. However, rifampicin markedly reduced the tizanidine/M-10 Ae ratio. Thus, it is possible that the formation of M-10 is partially mediated by a CYP-enzyme that is strongly induced by rifampicin, such as CYP3A4 [3, 4]. However, as rifampicin only slightly increased the Ae of M-10 and as only about 12% of the tizanidine dose is normally excreted in urine as M-10 [33], this finding alone cannot explain the 50% reduction in the AUC of tizanidine. However, it is possible that the reduction in the AUC of tizanidine by rifampicin was partially due to strong induction of an enzyme (e.g. CYP3A4) and metabolic pathway(s) with a minor contribution to the metabolism of tizanidine in non-induced subjects. This hypothesis could be tested by investigating the effects of selective CYP1A2 and CYP3A4 inhibitors on the pharmacokinetics of tizanidine in rifampicin-induced subjects.

In the present study, rifampicin reduced the AUC of tizanidine by about 50%, mainly by inducing its first-pass metabolism (no effect on $t_{½}$), and had only a minor, statistically non-significant effect (23%) on the caffeine test; rifampicin reduced the caffeine/paraxanthine ratio in eight of the ten subjects studied. The caffeine/paraxanthine ratio reflects mainly the systemic CYP1A2-mediated elimination of caffeine, since caffeine lacks presystemic metabolism [28–32]. The effect of rifampicin on the pharmacokinetics of tizanidine was, although small, still stronger than its effect on the caffeine test. Accordingly, it is possible that tizanidine could be more sensitive than caffeine as an indicator of CYP1A2 induction. It is also worth noting that the effects of CYP1A2 inhibitors on the AUC and C_{max} of tizanidine were greater than their effects on the caffeine test [35, 38, 51].

Only a 5-day pretreatment with rifampicin was used in the present study, and therefore hepatic enzyme induction

Table 4 Relationships between the pharmacokinetic variables of tizanidine and the plasma caffeine/paraxanthine concentration ratio during the placebo phase, and between their changes caused by rifampicin

Variable	Caffeine / paraxanthine ratio	
	r value	
	Value during placebo	Change caused by rifampicin
Plasma		
Tizanidine AUC(0–∞)	0.92***	0.75*
Tizanidine C_{max}	0.84**	0.69*
Tizanidine $t_{½}$	0.45	0.07
Tizanidine/M-3 AUC(0–∞) ratio	0.78**	0.65*
Tizanidine/M-4 AUC(0–∞) ratio	0.77**	0.63
Urine Ae(0–24)		
Tizanidine	0.92***	0.88***
M-3	0.83**	0.55
M-4	0.46	−0.09
M-5	0.89***	0.64*
M-9	−0.21	−0.19
M-10	0.28	0.45
Urine Ae ratio		
Tizanidine/M-3	0.86**	0.52
Tizanidine/M-4	0.97***	0.76*
Tizanidine/M-5	0.91***	0.73*
Tizanidine/M-9	0.93***	0.04
Tizanidine/M-10	0.82**	0.80**

AUC(0–∞) Area under the plasma concentration-time curve from time 0 to infinity, C_{max} peak concentration, $t_{½}$ half-life, *r value* Pearson correlation coefficient, *Ae* amount excreted in urine within 24 h
*$P<0.05$
**$P<0.01$
***$P<0.001$

was not necessarily maximal at the time of tizanidine intake [3]. However, an identical 5-day pretreatment with rifampicin has been shown to cause a very strong induction of CYP3A4-mediated metabolism [4–7]. Furthermore, Branch et al. [52] reported only a 16% increase in the paraxanthine-to-caffeine ratio in healthy subjects after a long, 28-day, treatment with 600 mg rifampicin daily. In other studies, rifampicin has modestly reduced the AUC of CYP1A2 substrates: orally administered theophylline (by 18–45%) [3, 10, 11] and caffeine (by about 40%) [12, 13], and intravenously administered ropivacaine (by 38–52%) [14]. Taken together, on the basis of the present results and previous findings, it seems obvious that rifampicin is at most only a weak inducer of CYP1A2-mediated presystemic and systemic metabolism in humans. Accordingly, drugs metabolised by CYP1A2 only are much less susceptible than substrates of CYP3A4 to drug interactions caused by enzyme inducers of the rifampicin type.

The effects of tizanidine on systolic and diastolic blood pressure and heart rate were weaker during the rifampicin phase than during the placebo phase, i.e. the reduced plasma concentrations of tizanidine had pharmacodynamic consequences. It is likely that the therapeutic effects of tizanidine are also similarly reduced during treatment with rifampicin, which may be of clinical significance in some patients. Notably, the main metabolites of tizanidine in plasma (M-3, M-4, M-10) lack pharmacological activity and have no clinical relevance [33].

In conclusion, treatment with 600 mg rifampicin daily moderately decreases the plasma concentrations of tizanidine, and slightly reduces its cardiovascular effects. The inducibility of CYP1A2-mediated presystemic (tizanidine) and systemic (tizanidine and caffeine) metabolism by usual therapeutic doses of rifampicin is weak at the most. Compared to CYP3A4 substrate drugs, substrates of CYP1A2 are much less susceptible to drug interactions caused by enzyme inducers of the rifampicin type.

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